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THE CROONIAN LECTURE

Transport of substances in plants

BY PROFESSOR H. H. DIXON, F.R.S.

(*Lecture delivered* 4 *November* 1937—*MS. received* 4 *November* 1937)

When the Royal Society—now many years ago—did the late Dr Joly and me the honour of publishing our first paper on the ascent of sap (Dixon and Joly 1894, 1895), it was not difficult to make a first approximation as to the mechanism, and the energy conditions, availed of by plants in lifting water from the soil to their leaves.

The driving force was to be sought in the vapour-pressure difference obtaining in the spaces surrounding the roots and the leaves (Dixon 1897).

A model was set up of two porous pots with a thermometer in each, connected hermetically with a glass tube. The whole was completely filled with water and set upright, one pot being buried in moistened earth—the other held aloft in the air. The arrangements are shown in fig. 1.

Here after a slight gravitational adjustment the liquid water is held in place by the capillary action of the menisci in the walls of the porous pots. Immediately the menisci in the upper pot, if surrounded by an unsaturated space, lose water and so reduce the water content of the artificial plant. This action draws in the menisci, and those in the lower pot being surrounded by the higher vapour pressure of the soil condense water upon themselves as quickly as water escapes from the upper menisci into the space of lower vapour pressure surrounding the upper pot.* Thus a flow of water molecules from the soil down the vapour-pressure gradient into the unsaturated space round the upper pot is maintained. The source of the energy expended in doing this work, i.e. lifting the water from the soil round the "root" of the artificial "plant" to the space round its "leaf", is revealed by comparison of the two thermometers. The lower one indicates a temperature sensibly higher than the upper one. The cooling above due to the escape of the energized molecules causes an inflow of heat at the upper menisci by radiation and conduction. This maintains the "ascent of sap" in the model "plant".

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* In passing it may be noted that the mechanism automatically delays the drying out of the artificial plant. Diminution of the aqueous volume leads to the incurving of the menisci. This in the upper pot checks evaporation, but in the lower one it favours condensation.

This artificial plant works extremely well if we do not lengthen its stem beyond moderation. But if we were to give it the dimensions of a forest tree, difficulties would arise immediately. It is evident that if the height of the water column connecting the two porous pots were to approach that of a water barometer, bubbles would begin to form in its upper end, owing to the reduced pressure there, and, breaking its continuity, cut off the supply of water from the upper pot.

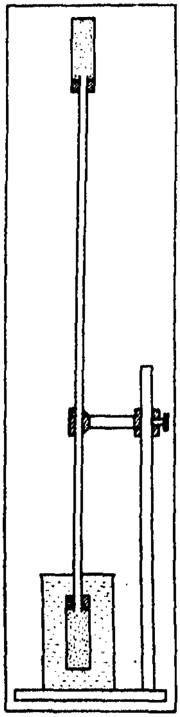

FIG. 1. Model representing the energy relations of the plant to its surroundings, two porous vessels connected by a glass tube. The lower vessel representing the root is plunged in damp earth, the upper one is exposed to the air. The difference in the state of saturation of the surrounding of each causes evaporation from the surface of the upper vessel and condensation on the lower.

To save the simile of the model "plant", it had to be imagined that the water in the tree, connecting the menisci in the roots with those in the leaves, has cohesion and tensile strength.

Water under ordinary conditions in glass tubes, as in our model, does not exhibit this cohesion; because there are suspended in it many unwetted particles, covered with films of air adhering to them, and similar films adhere to the inner surface of the glass. When the pressure is reduced, bubbles develop at these points, and as they expand preclude the water column from sustaining tension. But in the conducting tracts of plants a very different state of things exists, because the water passing into them has to traverse wet cellulose or woody walls. These wet walls permit the passage of water and *dissolved* substances, but are impassable for undissolved particles, whether they be solid, liquid or gaseous. Further, the walls being imbibed with water are completely wettable and afford no foothold for films of undissolved gas.

By washing with alkalis and repeated boiling we may wet the surfaces in masses of water, and thus, even in glass, submit water to tension and reveal its cohesion.

These methods show that under suitable conditions water exhibits tensile strength. They give no measure of this strength. Direct measurements are evidently of interest, and may be obtained by observing the dilatation on cooling of cohesive water, which has been made to fill completely thick-walled vessels by heating; or, by comparing the deformation of the containing vessels produced by the tension with that caused by known external pressures. By such experiments it has been shown that water, or sap centrifuged from the wood of trees, can resist tensions of 200 or even 280 atm. (Dixon 1909, 1914*a*). More recently, Renner (1915*a*), and a little later Ursprung (1915*a*), have most ingeniously used the breaking of the water in the cells of the annulus of the fern sporangium to determine this breaking strength; and, by balancing it against a known external osmotic pressure, have found that in these cells it amounts to at least 300–315 atm. Theoretical considerations lead one to expect a much higher breaking strength for water than is given even by these experiments.

Contrary to frequent and even recent statements on the matter the presence of *dissolved* gas introduces no instability, and here I wish to repeat with emphasis that Berthelot's description (Berthelot 1850) of his pioneer experiments is quite clear on this point, which Dr Joly and I further confirmed by repeated experiments (Dixon 1897, 1914*a*; Dixon and Joly 1895).

When first we propounded the view that water passed up through the conduits of trees in a tensile state, hanging by its own cohesion from the evaporating menisci, few people took the idea seriously; and uncomplimentary things were said of the theory and of those who suggested it. This was due to several causes, viz.: (1) Botanists were quite ignorant of the conditions under which the cohesion of water is exhibited. (2) There was a prevailing belief that the conducting tracts of plants normally contained air bubbles. This view that the tracheae contain air has been strangely nurtured by the very fact that they normally contain water in a state of tensile stress. For whenever a microscopic preparation is made to examine the contents of the tracheae, the mere excision of the material starts bubbles which remain in the tracheae to deceive the microscopist who believes in the evidence of his senses. (3) There was a fixed conviction handed down from the days of Donny (1846) that even traces of dissolved air reduced the cohesion of liquids to an insensible figure. (4) The difficulty of demonstrating to the unbeliever that the water in a transpiring stem is in a state of tension. So far as I know there is no visible distinguishing characteristic of water in the tensile state. The fact that the tracheae are strengthened to resist crushing or collapsing forces, and are unprotected against bursting ones (Dixon and Joly 1895) was not regarded as cogent evidence, and the inflexion of the walls between these supports and the contraction of the stems of transpiring plants, revealed

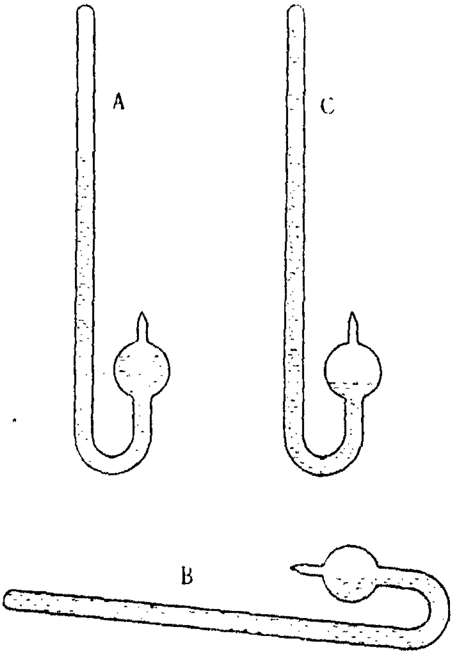

FIG. 2

FIG. 3

FIG. 2. Glass tube for demonstrating cohesion of liquids. *A*, position in which the liquid is not in tension; *B*, position in which the long limb is filled with liquid; *C*, position in which the liquid is in tension.

FIG. 3. Glass spherical vessel with flattened helical extension almost filled with cohesive water. By gentle heat the water is made to expand and to completely fill the sphere and the helix. When cooling sets in, tension in the contracting water increases the curvature of the helix.

by caliper measurements, in times of water shortage, were passed over lightly (fig. 4).

In recent years, however—now that we have become used to the idea of cohesion in liquids—evidence, which cannot be gainsaid, has been accumulated by the work principally of Renner (1911, 1912, 1915*a*), Nordhausen (1916), MacDougal (1921, 1929), Holle (1915) and Bode (1923). These investigators have shown that the rates of movement of water in cut branches are greater than can be accounted for by atmospheric pressure and must be explained by traction from above. They have demonstrated by measurements of the tracheae during transpiration that an internal tension is drawing their walls together. By multiplying levers they have made the contractions of the stem self-registering; and, above all, it has been shown that the majority if not all of the bubbles, observed in the tracheae, are artifacts, and that even wilted plants contain continuous water columns completely free from gas bubbles.

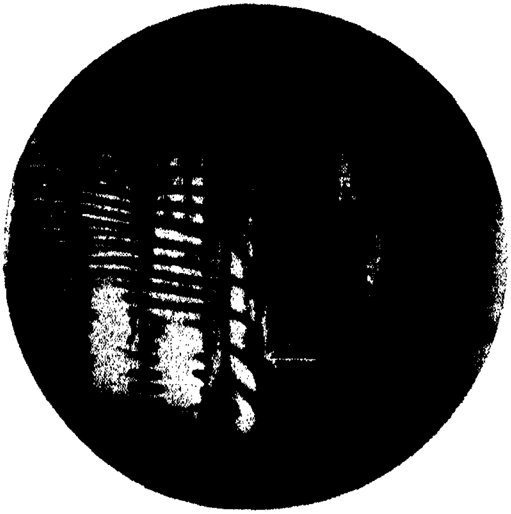

FIG. 4. Radial section of leaf bundle of *Ranunculus repens* fixed during transpiration × 100, showing the walls of the tubular tracheae drawn in between the internal supports.

Quite lately Dr L. A. T. Ballard and I have found suitable material for demonstrating very beautifully the tension developed in the sap during transpiration. The stem of a shoot of *Vitis striata*, supported in a vertical position, is clamped very tightly in a screw vice, so that the water supply coming from the roots is cut off from the shoot. Sooner or later its petioles and slender growing tips sag downwards. That this sagging is, in a large measure, due to the rising tension in the water conduits becomes immediately patent if the stem is cut under water above the clamp (fig. 5). For at that moment the petioles and tips of stems begin to rise, often moving through 90° in a few minutes. This elastic recovery may be readily studied and recorded by taking a series of photographs at short intervals from one another on the same sensitive plate (figs. 6 and 7).

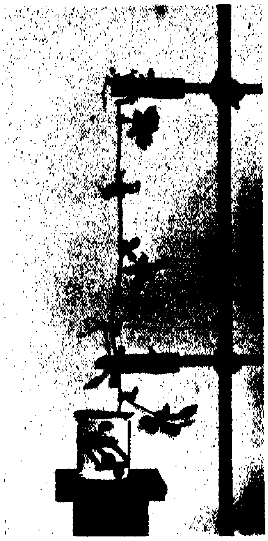

Fig. 5

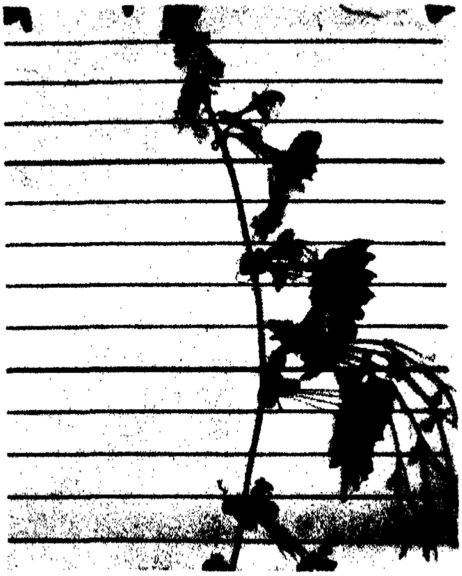

Fig. 6

Fig. 5. Branch of *Vitis striata* set up to show movements due to the release of tension. The beaker below contains the screw clamp still holding the cut off portion of the stem.

Fig. 6. Compound photograph, produced by four successive (40, 60, 60 secs. apart) exposures of the branch of *Vitis striata* showing the movement immediately following the cutting of the clamped stem under water. Movements of the longer petioles and of a side shoot are plainly recorded.

Here I would like to call attention to a very curious coincidence. In 1868 Herbert Spencer (1866) suggested a theory for the ascent of sap in plants. It depended on the fortuitous changes in volume of the tracheae owing to the swaying and bending of the stems and branches combined with the permeability of the tracheal walls. I do not think there is much to be said in favour of this theory, and it was presented without any experimental support; but the paper, which describes it, does contain a very remarkable sentence, which would almost make the modern reader think that Herbert Spencer was familiar with the much more recent cohesion theory. It runs as follows: "A state of capillary tension must result—a tendency of liquid to pass into the leaves, resisted below by liquid cohesion." The germ was there ready to develop, but the shell of the egg was never broken. My colleague, Professor J. McLean Thompson, called my attention to this remarkable utterance about twenty years after we had committed ourselves to the cohesion theory.

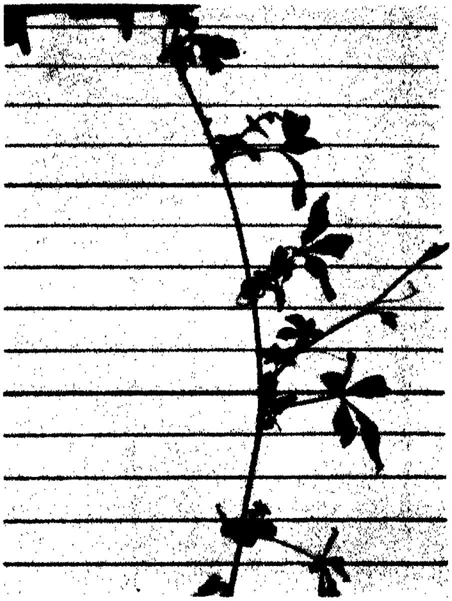

Fig. 7. A similar compound photograph of the same branch produced by four successive exposures made 20, 26, 32 and 38 min. after cutting the clamped branch under water.

In our model plant the evaporating menisci are supported in the walls of the upper porous pot. In the true plant, however, they are carried in the walls of cells which cover over the ends of the supply tubes. Indeed, the whole tracheal tissue (xylem or wood) which conveys the water through the plant is enclosed in a continuous sheet of cells (fig. 8). These cells are alive, and like living things have their secrets. They are of various shapes, but fit together without gaps, forming a covering to the tracheae, which precludes the entrance of undissolved gas. Each cell is a flexible bag of cellulose lined with a very thin film of living protoplasm and encloses a droplet of water, which though small (say 0·02 mm. in diameter) almost completely fills the cell. In this droplet of water carbohydrates and other solutes are dissolved. So in our imagination we must clothe our pots and tube with a covering of such cells and further suppose that the wall of the glass tube is permeable to water. To increase the verisimilitude of the artificial plant, we will further suppose that the cells covering the vertical sides of the tube have their outer walls covered with a waterproof film, while their inner and radial walls are of permeable cellulose. The walls of the cells covering the ends of the conduit may, in the first instance, be regarded as being quite permeable and free to lose, or gain, water in the form of vapour.

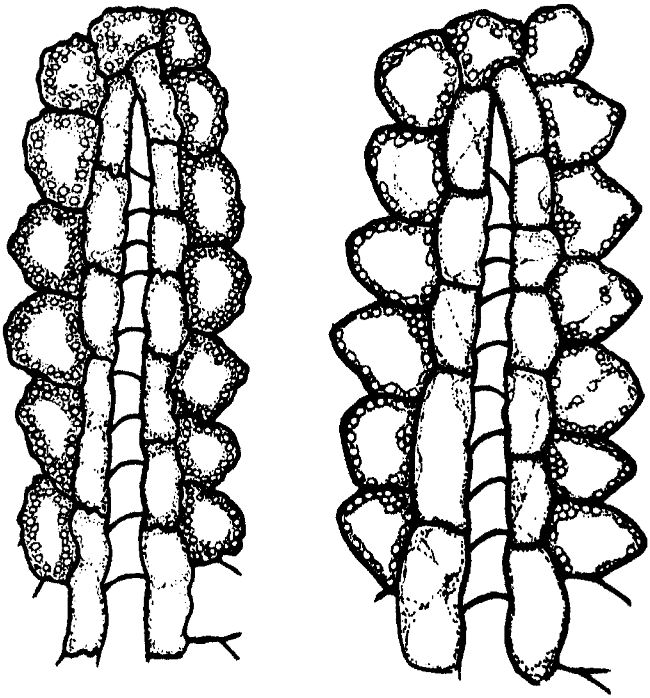

Fig. 8. Sketch showing the relations between the tracheal terminations and the covering cells in the leaf.

How will these cells affect the movement of water in the conduits?

An examination of the concentration of solutes in these cells shows that generally there is a greater concentration in the leaf cells of the plant than in those of the stem; and that those of the stem possess higher concentrations than those of the root; and on the whole a rising gradient of osmotic pressures in the cells surrounding the conduits has been found as we mount in the plant. Many exceptions to this general rule are recorded.

It was early suggested that the osmotic forces of these cells were available for lifting the water in the adjacent tracheae. De Vries (1884) and Pfeffer pointed out that the absorbent action of cells is not defined by their osmotic powers alone, but is only equal to the osmotic pressure less the inward component of the tension in their elastic cell walls. This available force drawing in water at any given state of distension was called the "suction force" or "suction pressure" of the cell.

Various methods have been devised for measuring suction force. In *general it has been found that there is a very regular gradient of suction* force in the cells adjoining the water conduits rising from below upwards in the plant. And to this gradient of suction force has been assigned, by many, on completely erroneous grounds, as will be seen, the function of lifting and of distributing the water in plants.*

There is no doubt, however, that in more or less wilted plants this suction force is operative in drawing water from the conduits into the flaccid cells; and if these cells are situated above and water is supplied below, this water is drawn up the conduits by the suction force of the expanding cells. At first there is little or no tension in the cell walls and the full osmotic pressure of the vacuole is available for drawing up the water. As the cells expand, however, tension begins to rise in their cell walls, and this tension acting against their increase in volume arrests their activity in raising the water in the conduits.

From this moment, when the steady state is attained, the net vapour pressure of the sap in the cell (primarily reduced by the solutes and subsequently raised, to some extent, by the tension in the cell wall) exactly balances the vapour pressure of the tensile water in the conduit at the level of the cell, and the movement due to the suction force ceases.†

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* To the gradient of suction force rising from the evaporating surfaces one general exception has been recorded by Ursprung and Blum (1916, 1921): that is, on the passage through the endodermis from the cortex of the root to the cells of the central cylinder within. Measurements here show that the cells on the inner side of the *endodermis have a markedly lower suction force than those of the cortex outside.* This is not surprising when we realize that the method of measurement used automatically releases the tension in the tracheae among the inner cells and they, being thus flooded with water, lose their pre-existing suction force. So this endodermis "jump" in the regular gradient of rising suction force (or declining vapour pressure) is to be regarded as an artifact unavoidable in the technique of the method of observation. It is interesting that these investigators, rejecting the cohesion theory, prefer to assume that the suction force in each endodermal cell is not homogeneous, but allows the cell to act as a suction pump on one side and as a force pump on the other.

† To speak of vapour pressure *in the body of a liquid, where there is actually* no free surface, is unusual. In the cases here considered it may be defined as the pressure of vapour in a bubble whose radius is such that its surface tension is in equilibrium with the hydrostatic tension at that level in the liquid.

The hydrostatic head and the friction opposed to the transpiration stream secures that there is a rise in tension in the water columns of the conduits from below upwards. This necessitates that the vapour pressure of the water in the conduits falls correspondingly; but, as the cells adjoining the conduits in the upper levels lose water to the conduits, till their vapour pressure falls to that in the conduits, it follows that the observed gradient of suction force is the necessary attendant of their proximity to the tensile water columns, and is determined by the gradient of tension in these conducting tracts. It is evident, then, that if there were a random distribution of various concentrations in these cells of a plant extended upon the ground, the mere elevation of the plant into a vertical position, by producing a gradient of tension and consequently of vapour pressures in the conducting tracts, would inevitably secure a parallel gradient of suction force in the adjoining cells. For into those cells, possessing too high a suction force, water would pass till the adjustments of tension in the walls and osmotic pressure in the vacuole produce a vapour pressure equal to that in the conduits at the appropriate level. Similar adjustments, in the converse sense, would take place in cells having a suction force too small for their level in the stem. So we may conclude that, no matter what was the distribution of suction force at the start, a gradient of suction force would shortly be established balancing the vapour pressures and tensions obtaining in the conduits throughout the plant. Hence we should regard these cells, not as engines doing the work of raising the sap, but as exquisite gauges automatically recording the gradient of vapour pressures and tensions in the tensile columns of water. And so the gradient of suction force contributes additional evidence in favour of the tension theory.

In the plant continuous water extends from the leaf cells to the root cells, and evaporation abstracting water molecules above tends to shorten the column which coheres with the menisci in the outer walls of the leaf cells. The tension so generated lowers the vapour pressure and a flow is maintained upwards, down the gradient of diminishing vapour pressure. This tensile column is continuous not only through the conduits formed of tracheae but also through the cells intervening between the evaporating surfaces and the water in the tracheae. The tension developed would tend to make these cells collapse and so diminish the evaporating surface, but it is resisted by the osmotic pressure of the solutes in the cells, which acting on the semipermeable protoplasm keeps these cells distended.

These conditions may be illustrated diagrammatically as in fig. 9. The youth is evaporation hauling on the tensile cords of water extending downwards through the tracheae of the xylem and through the cells, which afford the foothold for the evaporating surfaces. When tension is applied by evaporation the tracheae would collapse were it not for the strengthening action of their spiral and annular supports. Similarly, the evaporating cells, and the cells between them and the tracheae, would crumple under this tension were it not for the osmotic pressure of the solution which keeps them distended. But as we would not be justified in describing the compressions in the internal thickenings of the tracheae as forces raising the sap, so it is erroneous to attribute the normal lifting of the water in plants to the osmotic pressure of the cells adjacent to the tracheae or to their suction pressure (Dixon 1930).

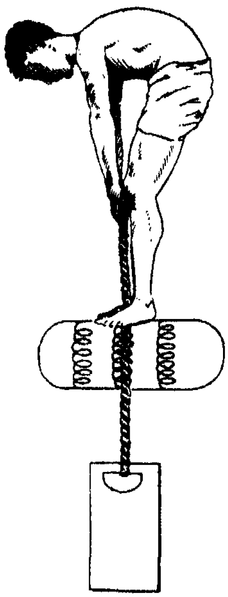

Fig. 9. Diagram constructed to illustrate the relations of evaporation (the youth) hauling on the tensile water column (the rope) to the osmotic pressure (the compressed springs) and the wall tension (tension in the fabric of the supporting cushion).

The interaction of the conditions of tension and osmotic pressure may be illustrated experimentally as follows: A glass tube 2 m. long and 5 mm. in diameter, closed at one end, is filled with a composite column of water and mercury. By suitable treatment the air films and unwetted particles are eliminated and a small piece of tissue, such as may be excised from the peduncle of *Doronicum austriacum*, is introduced into the water. One side of this piece is formed of the less extensible surface layers and the other of the ductile medullary cells of the peduncle. By repeated changes of cooled boiled water any chance bubbles or unwetted dust particles are removed from the piece of tissue before introduction into the tube. During this treatment the more ductile walls of the medullary cells yielding more to their osmotic pressures than those of the external layers cause the piece to become convexly curved on the medullary side. After the introduction of the piece of tissue the closed end of the tube is reared upright while the open end is kept immersed in a bowl of mercury. In this position the water rises to the top leaving the lower 160 cm. or so of tube filled with mercury. If all the discontinuities have been successfully removed and the liquid surfaces made to adhere to one another and to the glass, a tension of about 1 atm. is established in the water. This in no way destroys the osmotic pressure of the solutes in the cells of the enclosed tissue, which remains as before convexly curved although the liquid with which it is permeated is stretched with a tension of 1 atm.

The experiment suggests that if it were possible to move the piece of tissue, or a single cell of it, at will upwards and downwards in the tensile water, suitable adjustments would take place bringing its "suction force" into equilibrium with the vapour pressure of the level in which it finds itself. But its suction force could in no way be regarded as raising the column of water in the tube.

In this system, as in the plant, we may regard the solvent—water—as sustaining and transmitting the tension while the solutes continue to exercise their pressure directly on the protoplasm, and indirectly on the cell walls of the containing cells.

Discarding then the fallacious view that suction force normally raises the sap, can we suggest any satisfactory explanation of the varied magnitudes of the osmotic pressures found in plant cells?

Thin and permeable walls are obviously suitable for the gaseous interchanges necessary for actively metabolizing and evaporating cells. *In plants of simple organization*, such as herbs, the necessary rigidity is conferred on the whole body by the turgidity of the individual cells. Here the solutes in the cells supply the compression members, while the cell walls are the tension members of the whole more or less elastic, semi-rigid structure. The osmotic pressures (5–15 atm.) developed by these solutes are quite adequate to balance the tension necessary to overcome the weight of the water columns together with any resistance they experience being moved through the tracheal conduits. *In trees* the necessary rigidity is supplied by the framework of wood, but the increased length and height of the conduits necessitates greater tensions to raise the water. Hence we find much greater osmotic pressures in the leaf cells (20–40 atm.) which prevent the collapse of the evaporating cells when the tension across them has to equal the stress developed in the tracheae.

The mesophytic trees and herbs experience no particular difficulty in obtaining water from the soil. This is not the case with *true xerophytes*. The soil, in which their roots ramify, is usually extremely poor in water at one or more periods during their vegetative activity. This poverty is associated with a very low vapour pressure of water in the soil spaces. To obtain water under this condition the menisci in the walls of the root cells must have a still smaller vapour pressure. Hence the necessity of high concentrations in the sap (80–100 atm.). It should be noted that the low vapour pressures in the walls of the absorbing root cells may be produced by the concentration of their solutes, or by the tension transmitted to them from the leaf cells, rendering the menisci in their walls very re-entrant.

In contrast to these xerophytes, *the succulents* absorb water during periods of plenty. They have no great height or length of water columns; and so moderate concentrations suffice to keep their evaporating cells open for the transmission of the transpiration stream.

It should be realized, however, that often temporary and local metabolic processes supervene in these cells and completely mask the concentrations actually referable to these needs.

So far we have explained the upward movement of water from the soil to the leaves as essentially due to the gradient of vapour pressure. The whole has been regarded as a machine in which purely physical forces are developed. The fact that the osmotic pressures act upon living membranes seems accidental, and there appears no reason to suppose from what we have said that, if cells could be constructed with chemically precipitated semi-permeable membranes supported by cellulose walls capable of imbibing water, they would not function in the same manner as those in the living plant. Notwithstanding this it has been found that water does not rise in a dead plant, and its leaves dry. Merely killing the stem by heat or poisons need not, however, immediately cut off the water supply to the leaves; but the death of the leaves themselves quickly arrests the transpiration stream. It might be thought that these facts indicate that the arrest is due to the breaking down of the semi-permeable nature of the protoplasmic films in the evaporating cells—this change being known to supervene with the death of the protoplasm. Other facts, however, point to another conclusion.

The simply functioning machine depending on a gradient of vapour pressure, maintained by an inflow of energy at the cell surfaces in the leaf and transmitted by tension in the solvent through the cells and tracheae in its path to the root, would undoubtedly come to a stop if the evaporating cells were surrounded by a saturated atmosphere in which temperature conditions produced condensation on their surfaces. The surprise that so often awaits us in biological material now enters on the scene; under these conditions a plant does not necessarily cease transpiring.

The following simple experiment staged this *dénouement* for me: Leafy stems were severed from their roots by cutting under water and kept in saturated spaces with their cut end supplied with water for about 8 hours or more. Their cut ends were then submerged in a solution of eosine, and the branches thus supplied with eosine were set under a glass receiver together with a beaker of hot water. A suitable screen was arranged to cut off the direct radiation from the branches and the receiver was closed and set in a dark press (fig. 10). After a short time (15–30 min.) the receiver was opened and the parts of the stems submerged in the eosine solution cut off. Microscopic examination showed that in every case eosine had passed up the xylem of the experimental branches. This experiment described by me (1898) clearly demonstrates that water may be extracted from the upper ends of the conduits by the adjoining cells while water is being condensed upon the outer surface of these cells—a movement against a vapour-pressure gradient.

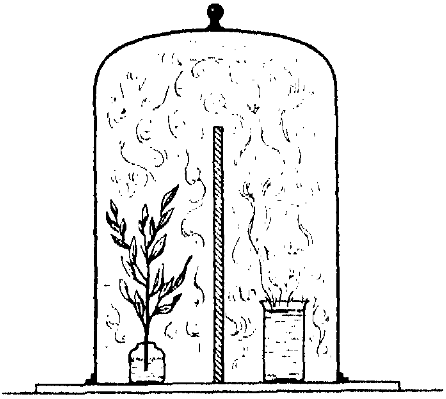

Fig. 10. Transpiration into a saturated atmosphere.

Another demonstration of the same action may be effected as follows: A leafy branch is cut under water and its cut end while still submerged is passed through a tightly fitting perforation in a rubber bung. This bung is now fixed in the neck of a receiver so that the cut end projects from the neck and the leafy part of the branch is enclosed in the receiver. The receiver is now set in a retort stand so that the cut end dips into a basin of water, and the receiver is kept saturated with water vapour. A fresh surface is cut on the submerged basal end of the branch and the whole is set in a dark press for 8–12 hr. At the end of this time the receiver is filled with water and the basin is replaced by a beaker containing a solution of eosine (fig. 11). It will now be found that the eosine mounts into the conduits of the branch and passes out into the leaves. This rise is much accelerated when the apparatus is exposed to sunlight. The rise does not take place, or is negligible, if the receiver is filled with cold boiled water and set in the dark.*

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* Since this was written Dr P. G. Mason has drawn my attention to a paper by Smith, Dustman and Shull (*Bot. Gaz.* 1931, p. 395) who urge that in my experiments the rise of the dye was due to the unsatisfied water-deficit of the branches and

We conclude from these experiments that the cells at the upper ends of the conduits secrete water on their surfaces which they abstract from the conduits, and that they may do this even into a saturated space. The experiments also afford evidence that the work of separating the water from the solutions in the vacuoles is done at the expense of the respiratory energy liberated in these cells. The action is probably quite similar to the secretion of water by the coenocytes of many of the fungi from watery substrates, and to the exudation of water drops from the ends of grass leaves.

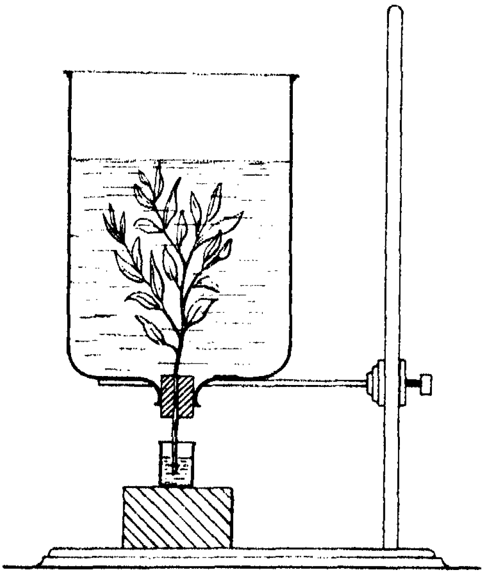

FIG. 11. Subaqueous transpiration.

The foregoing considerations have led us some distance from our first model illustrating the mechanism by which water is raised in plants; and, according to the modifications we have had to assume, we now view the plant's mechanism as somewhat more complex. The whole may be regarded approximately as follows: In the fine-grained walls of the cells abutting

leaves; and state that they could find no such rise when this deficit was removed by complete submergence of the material for 24 hours previous to the experiment. Such prolonged submergence, unless asphyxiation were guarded against, would of course tend to put any respiratory process out of action. In my early experiments the saturation of the branches and leaves was effected by their enclosure in a dark saturated space with their cut ends standing in water. In experiments recently carried out at low temperatures (9·5° C.) and on rather weakly material, I have obtained moderate rises in branches of *Chrysanthemum sinense* which had been totally submerged in aerated water for 24 hours.

on the intercellular spaces of the leaves are water films which have been formed of water separated from the solutions in the vacuoles. The energy for this separation is supplied by the inflow of heat at the menisci in the cell walls establishing a tension in the solvent and drawing it outwards to make up for the loss of water molecules abstracted as vapour. This action of heat separating water from the solutions may be replaced, or assisted, by respiratory energy in the cells. The tension established in this way in the solvent is transmitted, it may be through one, or through many cells to the purer water in the tracheal conduits. These cells do not collapse but retain their normal surface and full rounded contours by reason of the strutt-like action of their contained solutes. The tension of the solvent—water—is transmitted to the contents of the tracheae, which are similarly protected from collapse by the action of their thickenings (spiral and other), and further to the solvent in the root cells and to the water films in their walls. Here the draught upon the solvent produces menisci with very small radii of curvature and consequently very low vapour pressure. Into these stream the vapour molecules, or the free water, more or less filling the interstices of the soil. Meanwhile the tension in the solvent, continuous throughout the tracheae and adjoining cells, secures that the difference between the osmotic pressure and the tension in the cell wall ("suction force") exactly balances the vapour pressure in the tracheae at the corresponding heights in the plant. And this tension gradient extending into the continuous solvent, which permeates the trains of cells lying between the tracheae and the plant surfaces, develops in these cells two parallel gradients of declining vapour pressure and consequently of mounting "suction force".

The transpiration stream is a dilute solution. It contains salts, acids, carbohydrates and even enzymes (Wotchal 1915), as may be judged from chemical, cryoscopic and conductivity experiments on the liquid centrifuged from segments of stems and roots (Atkins 1916; Dixon and Atkins 1910). The transverse walls are freely permeable to dissolved crystalloids, and consequently the stream, as it moves, carries with it these dissolved substances. Percolation experiments confirm this, and it is found that these solutions pass upwards or downwards through the xylem of trees sensibly unaltered in concentration, showing that the partitions exercise no filtering action so far as these substances are concerned.

Normally this stream moves upwards and carries its solutes with it; and in tracing the transport of water and its mechanism we are at the same time revealing the manner in which solutes travel. The motion is down the gradient of aqueous vapour pressure from the usual *source* of higher vapour pressure—the soil—to the space surrounding the leaves having a lower vapour pressure, and this space is the *sink* towards which the water and its solutes make their way. We have seen that this gradient of vapour pressure is on occasions maintained or controlled by the living cells of the leaves; so it is quite reasonable to conclude that cells adjacent to the tracheae of the xylem, by adjustments in their secretory activities, are able to abstract dissolved substances from, or introduce them into the transpiration stream.

Another factor must have a controlling influence on the direction of transport. That is, the permeability of the tissues adjoining the tracheal conduits. It is well known that as a general rule the films of protoplasm enclosing the vacuoles and lining the cell walls are highly impermeable to the solutes of the vacuolar sap. This is evidenced by the high electrical resistance manifested by these tissues and by other means of testing their permeability. Exposure to anæsthetics or a rise of temperature, however, greatly enhances the permeability or conductivity of these tissues (Dixon 1914*a*, *b*, 1924; Dixon and Bonnet-Clark 1928) (fig. 12).

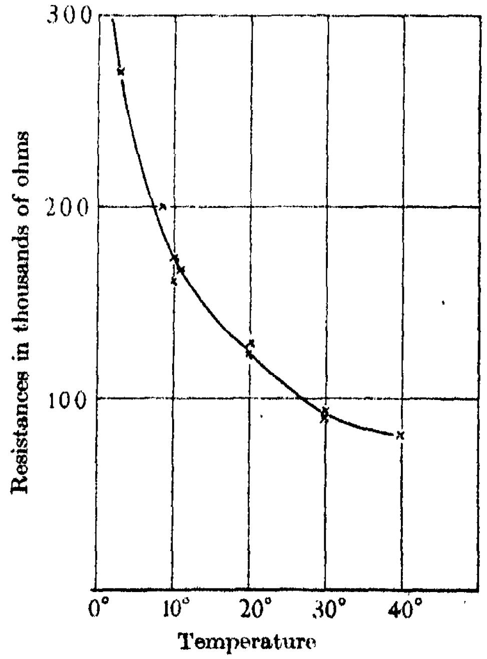

Fig. 12. Relation between the resistance of leaf-tissues and temperature. Each point is the average of 18 determinations. 28/11–10/12/'27.

We have only to imagine two such regions in the leaves, or one in the leaves and one in the roots, connected by a tensile column of water in the tracheae. Normally, evaporation from, or the secretory activity of, the upper region makes it the sink; but exposure to sun heat may render its protoplasm more permeable than that of the lower one, which remains a steady basis of attachment for the contracting column and thus is able to draw out the solutions of the upper cells through their more permeable protoplasm into the adjacent tracheae (fig. 13). Changes of temperature may at times restore the original permeability, and metabolism rehabilitating the osmotic pressure may replenish the depleted cell or tissue to its original volume. Repetition of this cycle will produce a more or less satisfactory, if fortuitous transport system in the plant through the wood or xylem (Dixon 1922).

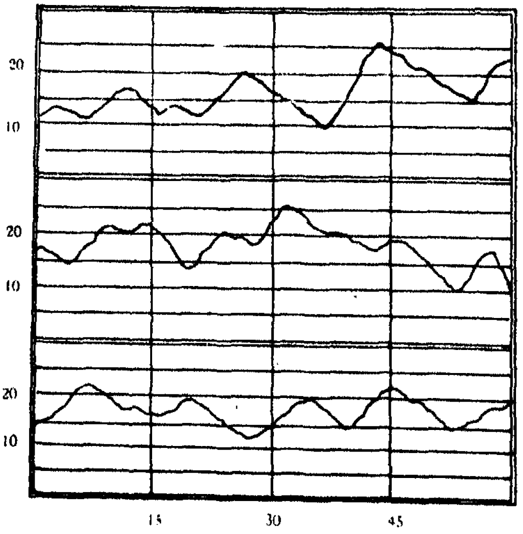

FIG. 13. Three graphs showing changes in temperature differences between two regions in the same leaf of *Syringa vulgaris* exposed to alternating sun and shade on a breezy day. Ordinates in 0·1° C. and abscissae in seconds.

Once the solutes are in the tracheae the adjoining cells are free to exert their powers of selective absorption and take in what solutes are needed in their metabolism.

The interchange of source and sink can be easily realized experimentally. If a leaf of a rooted and transpiring potato plant be cut under the surface of a solution of eosine, the tip of the leaf, which up till then was the sink towards which the tensile sap was being pulled, suddenly becomes a source by the inrush of the eosine drawn in by the tensile sap. The movement of the liquid supplied from the source is continued by the elastic expansion of the tracheae and of the tissues surrounding them as they are released from the original tension in the sap. Meanwhile the leaves of the other haulms are exerting their traction upon the water in the tuber, and it, by this means, becomes a sink towards which the eosine is drawn. Later on when the tracheae of the tuber are filled they in turn act as a source for the leaves on the uninjured haulms, and so the whole tracheal system in the tuber and in the haulms becomes injected with the coloured liquid (Dixon 1922).

But by far the most striking transport of solutes in the tracheal tissue is that which occurs during the unfolding of the leaves of deciduous trees and shrubs and in some other plants. During this period the concentration of sugars in the sap is so high (20–33 % according to Hartig 1858, 1861) that even in remote times the constitution of the sap attracted attention, and it has long been used in the production of alcoholic drinks. It is true that after the expansion of the leaves the tracheal sap usually becomes much more dilute because of the great influx of water from the roots to swell the transpiration current; but still even then it must have considerable importance in the general transport of sugars and other solutes in the plant.

Having regard to this available and certainly, at times, highly effective system, I was inclined (Dixon 1922) for a time to doubt the necessity of assigning any great importance to the phloem, or conducting tissue of the bast, in the transport of organic substances, more especially as the experimental evidence for its being the channel for their conveyance was at that time very unsatisfactory. Now, however, the aspect of this evidence has been completely changed by the brilliant work of Mason and his collaborators (Mason and Maskell 1928, 1929, 1930, 1931). These workers have shown by the unequivocal chemical results of convincingly planned ringing experiments that there is an important movement of carbohydrates and of other solutes in the phloem. The evidence is very clear that the direction of the movement of the carbohydrates is down the gradient of concentration. For the other solutes the evidence is not so cogent.

The mechanism of this transport in the phloem is at present unknown and affords a most attractive field for research and speculation. The velocity of the movement is some tens of thousands of times too fast to allow it to be explained by the observed gradient and the simple laws of diffusion. Mason and his co-workers suggest an accelerated diffusion. Curtis (1935), reviving an old idea of de Vries (1885), would call in the assistance of the streaming of the protoplasm in the elements of the phloem to bring about this acceleration. But while streaming may be observed in the immature and non-functioning elements of the phloem, it apparently does not occur in the mature ones—just where it is wanted. Again, the streaming could at most contribute a factor of 1000, while what is required is one of 20,000.

At present there seems to be only one other hypothesis left, i.e. that of differential osmosis, which is usually attributed to Münch (1926, 1930 and Nordhausen 1916). This theory, as I understand it, supposes that the elements of the lower phloem and their adjacent cells abstract, or render insoluble, some of the solutes in the phloem, thereby raising the vapour pressure of the water in the phloem and causing it to pass into the adjacent tracheae where the vapour pressure is less (owing chiefly to tension). There it passes into the ascending transpiration stream. Part of this stream following the vapour pressure gradient enters the ultimate extensions of the phloem in the leaves, and increasing the bulk of the osmotic solutions in the upper parts of the phloem keeps up the circulation by forcing down the contents of the phloem from above, and thus a mass movement downwards is produced. The very low vapour pressure in the upper phloem, which is a necessary link in this chain, is maintained by evaporation and photosynthesis; and we must suppose that respiratory energy enters the system where the phloem cells adjoining the xylem separate solutes from the solution and also where solutes are introduced into the upper phloem.

It must be freely admitted that the results of Mason and his collaborators indicating that the individual solutes are amenable solely to their own particular concentration gradients, if substantiated, are against the theory of mass movement of the mixed solution in the phloem.

I have already published (Dixon 1933) figures indicating that the concentrations of the bast sap at different levels support this theory; and lately (Dixon 1937), by tracing the convection of heat in stems (fig. 14), I have obtained clear evidence that, while the transpiration stream is moving upwards, there is often another stream which is moving simultaneously downwards (fig. 15). This may well be the looked for mass movement in the bast; but on the other hand, reversals of water movement in the tracheal conduits are not excluded.

At the moment feeling our way among these puzzlingly contradictory observations we may be in "outer darkness" or within one step of the light.

In this connexion we naturally think of another enigma in the transport of material in plants. In recent years nothing has excited physiological botanists more than the discoveries regarding the growth substances in plants, and not the least remarkable of these are the facts which have come to light with regard to their transport.

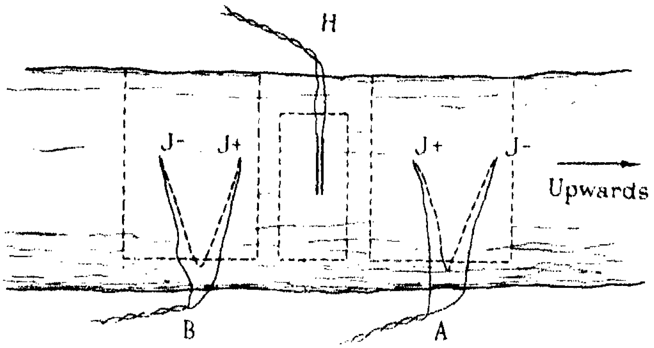

FIG. 14. Diagram showing the arrangement for observing the convection of heat in a branch. *H*, electric heater; *A* and *B*, thermocouples above and below the heater. The connexions were so arranged that when the junctions ($J+$) nearer to the heater were hotter than those ($J-$) further from it, the galvanometer gave a positive deflexion. The lagging and weather protections covering the junctions and heater are not shown.

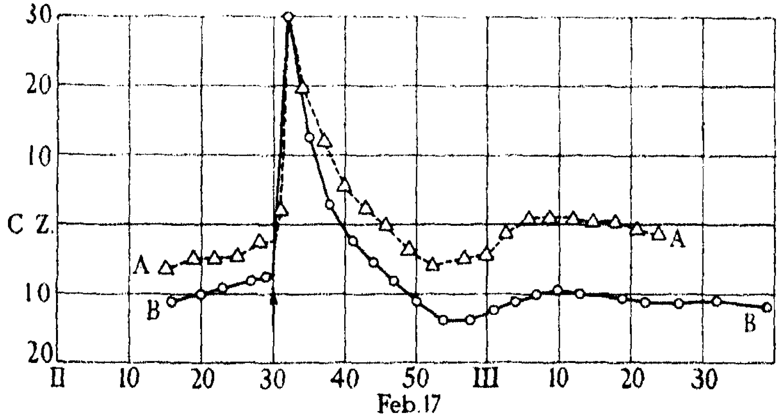

FIG. 15. Sample graph of the simultaneous galvanometer deflexions due to the temperature differences of the couples (A) above and (B) below the heater. The ordinates are the deflexions in mm. and the abscissae the times in minutes. The vertical arrow indicates the moment when the heater circuit was closed for 10 seconds. The fall of the curves below the level indicated at the moment of heating shows that heat has been *conveyed* from ($J+$) to ($J-$) and that hence mass movement has taken place in the stem in those directions.

These substances have been found to move from the seat of their production to the tissue stimulated by them at the velocity of about 10 mm./hr.—much faster than can be accounted for by diffusion. Unlike the transport of the solutes in the phloem, these substances, travelling in the cells of the cortex, move apparently regardless of their concentration gradients, but always in a basal direction. And a high concentration at the basal end of an experimental organ does not impede movement down to it from a supply of lower concentration. Gravity and light appear to exercise a slight directional force. Furthermore, while temperature seems to exercise little effect on the velocity of the movement, a rise in temperature is reported to increase the amount transported. In addition to this polar transport through tissues formed of living cells, growth substances applied to the roots are carried upwards, like the carbohydrates, in the transpiration stream. Diffusion, protoplasmic streaming, surface tension and electrophoresis have been invoked in vain to supply a satisfactory explanation of the transport of growth substances.

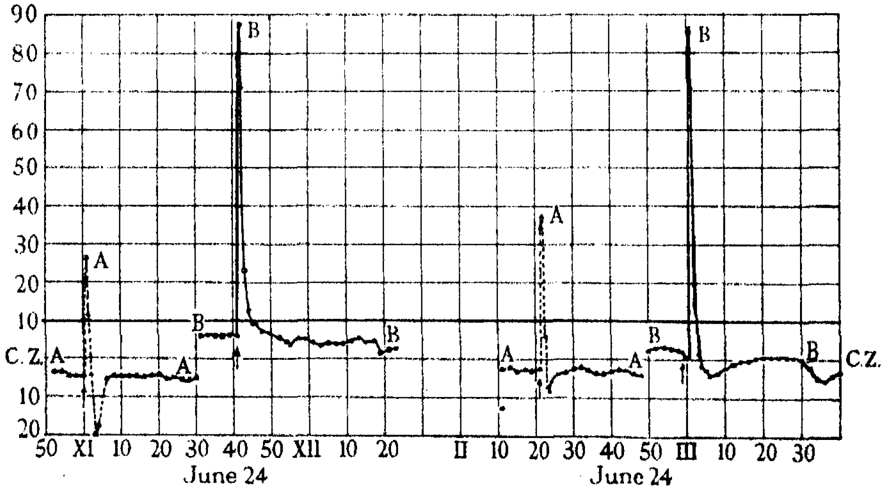

FIG. 16. Similar thermocouple records obtained in summer, indicating convection of heat (and therefore mass movement) downwards as well as upwards in the stem of *Fraxinus excelsior*. The lettered references are the same as in fig. 15.

In another branch of recent botanical research the problem of transport has become urgent. During the last year it has been found possible to produce the pathological symptoms of certain virus diseases by the injection into healthy plants of proteins in a highly purified state isolated from diseased plants (Stanley 1934*a*, *b*, 1935*a*, *b*, *c*, 1936; Bawden and Pirie 1937). In this way a speculation, expressed by myself many years ago, and possibly by others too, has been rendered highly probable, viz. that viruses should be regarded not as specific submicroscopic organisms, but rather as specific substances, which do not reproduce themselves as organisms, but rather catalyse transformations of pre-existing materials so that as a result additional molecules of the virus are produced. This view seemed to me to fit the observations then available, viz.: (1) the absence of a limiting surface between the virus and the protoplasm of the host; (2) its rapid increase in its host; (3) the vanishingly small quantity of the virus needed for inoculation; and (4) what interests us most now, considering problems of transport, the rapid transmission of the symptoms of these diseases in the tissues of the host amounting to 10–30 cm./hr. (Severin 1924; Storey 1926). It is further reported that viruses spread most rapidly from growing tissues, where presumably the subunits of the protoplasmic proteins are in great abundance, and that they travel by way of the plasmodesmata, while their path is blocked by dead cells and unperforated cell walls.

A beautiful example of the transmission of such an autocatalytic formation was brought to my notice many years ago by the late Dr Joly. It is the spread of crystallization in the supercooled solution of sodium acetate. The solution is saturated with the salt when surrounded with boiling water and is introduced into a straight or spiral tube. The complete solution of the salt is then secured by the immersion of the tubes for some time in boiling water. After this the tubes are allowed to cool to air temperature. Thus cooled the solution may remain for hours, or days, as a clear liquid—but at any time crystallization may be started by chance—or with certainty, by inoculating a single minute crystal of the salt into the clear liquid. From this nucleus crystallization spreads downwards at a velocity of about 40 cm./min. (fig. 17).

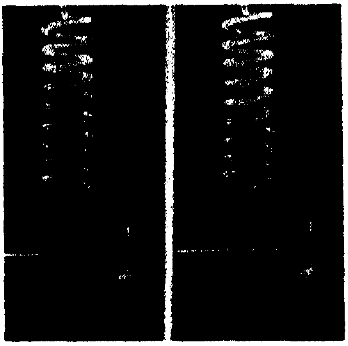
(*a*) (*b*)

FIG. 17. Two photographs taken successively with an interval of two minutes between, showing the advance of crystallization of sodium acetate solution in a spiral tube.

If the suggestion that the movement of the virus is comparable to this advance of autocatalysis is established, then the advance of pathological symptoms associated with the virus is not due to the transport of matter but to the advance of a change of state of the matter already pre-existing in the path traversed.

In conclusion I would like to acknowledge the help given me in preparing the illustrations by Dr L. A. J. Ballard, and in taking the photographs by Mr J. Murray.

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Dominance in poultry
Feathered feet, Rose comb, Internal Pigment and Pile

By R. A. Fisher, F.R.S.

(*Received* 27 *September* 1937)

1. Introduction

In a previous report (Fisher 1935) I have described the results of an experiment with poultry, covering the years 1929–34, so far as these concerned the three factors for Crest, Polydactyly, and Barred plumage. It was hoped at that time that two further years' experimentation would enable the conclusions to be demonstrated decisively by the production of parallel broods consisting exclusively of homozygotes and heterozygotes, in which the differences between these genotypes, and the variability of each, could be directly observed. This would require at least one male and several female homozygotes of the same kind, material which I have not succeeded in breeding in any line.

Although the stock did well in 1934, the year in which in most lines intercrossing was first practised, the two following years were exceedingly unfavourable to propagation. In 1935, for example, from over fifty sittings of eggs, only fifteen chicks in all were reared to maturity. Little of consequence can, therefore, be added in respect of the three factors previously dealt with, and the present report will be given principally to the four remaining factors.

Two small broods were indeed raised in 1935 from a herniated hen, crossed with an uncrested cock, and from these the two birds which lived long enough developed crests, while six others, having single combs, could be diagnosed as Crested owing to the combs being abbreviated and bent posteriorly in a way which appears to be characteristic of single-combed Crested birds (Brandt 1936). There can be no doubt, therefore, that the herniated mother, as anticipated, was homozygous for Crest.

The homozygous Barred cock, bred in 1934, mated to barred hens, produced a brood of only two chicks, of which the one male was homozygous like his father, while the female also was barred.

Birds heterozygous for polydactyly have, in these experiments, so frequently shown normal feet that a test on no larger scale than these was bound to be inconclusive. Of the eleven chicks, bred from apparently homozygous mothers, no less than seven were four-toed, and none of these survived to provide the opportunity of showing that they carried the gene for polydactyly. The analogy of the cases of Feathered feet and of Rumplessness, for which larger numbers are, fortunately, available, and which are discussed below, suggests that in this case, also, we are dealing with no more than a failure to manifest the heterozygous condition in a high proportion of the small number of chicks which it was possible to breed.

We may now consider the results for the four remaining factors, Feathered feet, Rose comb, Black Internal pigment and Pile plumage, which, in the course of the experiment, were introduced into a stock of wild *Gallus gallus*. A brief summary of the entire results is given at the end of this paper (p. 46).

2. FEATHERED FEET

The four Silky pullets used in 1929, for the primary cross, had feathered feet, but apparently were not all homozygous, for, out of thirty-four chicks bred, fourteen were unfeathered. Of the nine cross-bred pullets used in 1930 six were chosen as having feathered feet, but their progeny was not kept separate. Of the 144 chicks bred, forty-one showed feathering. Two of these were crossed in 1931 with a wild cock, and the character continued to be readily classifiable at hatching. The author was, however, away for some months during this summer, and, on his return, birds with feathered feet could not be distinguished with certainty from their normal sisters. Consequently, in 1932, all surviving pullets from this pen were mated for a fourth crossing to the wild stock. In 1932 only a quarter of the chicks were expected to be feathered, and in fact the feathering was recovered in six out of thirty chicks. Two of the thirty, including one feathered cockerel, were polydactylous, showing that polydactyly had been carried by one of the 1931 birds in this pen.

Since the gene for feathered feet seemed in the wild stock to be already nearly recessive, it was thought desirable to obtain homozygotes without delay, although the extent of feathering was still very variable. In 1933, therefore, a feathered cock was mated to two feathered hens, none of these showing polydactyly. Again, one at least of the parents must have carried this factor, for it reappeared in the chicks from this mating.

Of the nineteen chicks bred, only three were without feather. Of the remainder, some showed the very weak and transient feathering which had given trouble in previous years, some a medium degree of feathering, while in others it was very strongly developed. These latter, it was noticed, displayed additional features distinguishing them from birds showing the medium degree of feathering. In the first place they were brachydactylous, having the fourth toe noticeably shorter than the second. The claw in particular of the fourth toe was dwarfed, and on examination it was found that the toe itself contained three phalanges instead of the normal four. The third phalanx, moreover, was the smallest of the series, contrary to what occurs in other cases when the third and fourth phalanges are fused to form a single bone.

The second characteristic feature was that the line of feathers which passed obliquely across the back of the foot, instead of terminating at the base of the fourth toe, was extended up to the second or third joint. One of the chicks which showed these features died in the shell, and three more during the first few weeks of life. Two, however, from a late brood (29 July) seemed likely to live. They were both females, and in them it was noticed that the feathering was persistent, and not soft, but bristly to the touch. One of these pullets died in November and the other, which was also polydactylous and Silky, broke her leg and had to be killed during the winter. She had always been lame, and the accident was doubtless due to the crippled condition of her feet.

There can be now little doubt that the six birds from this mating showing the three combined characters of heavy feathering, extended feathering and brachydactyly were homozygotes for the gene for feathering. As, however, none of them survived, it was necessary to repeat the experiment in the following year. The 1933 broods had, however, shown that the attempt to obtain homozygotes in that year was somewhat premature; the strain had been by no means freed from factors introduced in the original Silky parent. Apart from the appearance of Polydactyly, recessive factors for Silky plumage and for Black plumage also made their appearance. With so much germinal material present of domesticated origin it was to be anticipated that the strain would be far from pure also in factors affecting the manifestation of the gene for feathered feet.

In 1934 two of the cocks from 1933 were tested by outcrossing and both were shown to be heterozygous. One of them, a weakly feathered male, was also mated with two weakly feathered sisters and gave a brood of six, four strongly feathered, one weakly, and one without feathers. Of the four strongly feathered, one, a Silky, died at 10 weeks old, but the remaining three, a cock and two hens, appeared to be typical homozygotes, showing brachydactyly and extension of the feathering with its stiff bristle-like texture. The male, unfortunately, died during the winter, but the two females, one of which was polydactylous, were available for mating in 1935 and 1936.

With males from lines from which feathering was absent, they gave the numbers shown in Table I.

TABLE I. CLASSIFICATION OF CHICKS FROM HOMOZYGOUS MOTHERS

		F	+
1935		3	1
1936	H	7	0
	O	0	1
	U	7	3
	Bb	9	0
	Total	26	5

The great excess of chicks with feathered feet shows that the mothers are not segregating in the ratio 1 : 1 as they would do if they were heterozygous. The presence of five chicks with unfeathered feet does, however, require explanation. In both years it was observed that some of the chicks, though classified soon after hatching, showed feathering in the minimal degree capable of detection, namely, a single down feather on one foot only, the other foot being unfeathered. With manifestation at this level it is not improbable that at least as many chicks carrying the feathered gene should fail to show any phenotypic effect whatever. The gene for feathered feet is, in fact, behaving almost as much like a recessive as a partial dominant, and may completely fail to show itself, just as polydactyly has been shown in this same line to do. A parallel back-cross in 1936 with hens classified as heterozygous gave, in fact, seven feathered to nine normal, numbers which, apart from emphasizing the contrast with those judged to be homozygous, show a small excess among the unfeathered.

In respect of modification of dominance, the feathered line yielded some other interesting results. One of the cocks outcrossed in 1934 was found to give chicks rather strongly feathered, with the third and fourth phalanges of the fourth toe fused, as though displaying an initial stage of brachydactyly. Such an effect on heterozygotes, causing them somewhat to resemble the homozygote, is exactly what should be expected on the view that dominance is very liable to modification. The matings of this cock with heterozygous mates are summarized in Table II, in which distinction is drawn between the two types of heterozygotes, the strongly feathered with fused phalanges, and the weakly feathered.

TABLE II. MATINGS SHOWING TWO SORTS OF HETEROZYGOTES

	Brood	No feathers	Weakly feathered	Somewhat strongly feathered, fused phalanges	Very strongly feathered, extended, brachydactylous
1934	**J**	1	1	—	4
1935	**G**	—	1	4	—
	S	3	—	2	—
	W	1	3	2	2
	Bb	6	—	3	2
	Total	10	4	11	4
1936	**B**	1	—	3	1
	T	5	3	4	2
	Total	6	3	7	3
3 years		17	8	18	11

The supposition of a single modifying factor carried by the male only would give an expectation of 13·5 in each class. The deviations above give $\chi^2 = 5{\cdot}111$ for 3 degrees of freedom, which is not significant. The excess of strongly feathered heterozygotes does, however, suggest that one of the hens may have carried the modifier. We should expect, however, that the weakly feathered class would be depleted by the classification of several of the weak heterozygotes as non-feathered.

Summary for feathered feet

In wild stock the mutation for feathered feet shows itself in the heterozygote by a very weak and transient feathering. This may usually be recognized at hatching, but is often imperceptible later.

The homozygotes show a strong feathering, extended up the fourth digit, retained to adult life, bristly in texture, and accompanied by brachydactylous (three-jointed) fourth toes. The third joint and the claw are especially dwarfed. Occasional feathers sometimes appear on the distal joint of the third toe.

The mutation is thus nearly, but not quite, recessive. In comparison with the nearly recessive mutant Barred, Feathered feet seems slightly the more recessive.

Although the experiment was not sufficiently extensive, and was not carried far enough to analyse the causes of variation among heterozygotes, the observations agree in detail with the view that one dominance modifier from the domestic strain was still segregating in some of the test progenies.

3. SUPPRESSION OF THE HETEROZYGOUS AND CHANGE OF THE HOMOZYGOUS MANIFESTATION OF AN INCOMPLETELY DOMINANT GENE

Extensive breeding experiments reported by Dunn and Landauer with the mutant for Rumplessness have revealed a series of remarkable facts bearing on the modification of both heterozygotes and homozygotes. The mutant had at first been described as "dominant"; Dunn and Landauer found in the stocks first examined that the term was apparently justified:

> In our original material and in the experience of other investigators this gene behaved as a "dominant". Fowls heterozygous for the gene lacked the free caudal vertebrae, one or two synsacral vertebrae, the fleshy rump, the tail feathers and the uropygial gland. This condition, which was likewise characteristic of the few known homozygotes examined, we referred to as complete rumplessness.

When this stock was outcrossed to normal breeds, dominance tended to disappear, and intermediate heterozygotes began to predominate.

> The factors responsible for these modifications towards normal proved to be hereditary, and after selection and inbreeding among members of the modified stock, ratios approaching ¼ complete rumpless : ½ intermediate rumpless : ¼ normal were obtained from *inter se* matings of intermediates; while from matings of intermediates by normals from the same stock there resulted chiefly normals and intermediates, with very few complete rumpless.

By an outcross to a non-rumpless breed, followed by selection, Dunn and Landauer had produced a condition closely analogous to that of Crest, Pile, Polydactyly, Feathered feet, etc., when mated repeatedly back to wild stock.

The results from 1927 to 1933 led these authors to think that the modifying factors affected only the heterozygotes. Two further generations of selection, however, showed that some of the homozygotes also were beginning to show "intermediate" features. Thus, in a few generations of selection, Dunn and Landauer have not only demonstrated the modificability of dominance by selection, but have gone far towards verifying also the further inference of my 1928 paper (p. 123):

> It is clear, however, that a persistent mutation in which even the homozygote has not too bad a chance of survival, the homozygote may follow in the footsteps of the heterozygote, and become indistinguishable from the wild form.

Dunn and Landauer do not, in their report, call attention to the circumstance to which the rapid success of their experiment in selection is doubtless due, namely, that the gene complex, in which the mutant for Rumpless shows no dominance, is that normally present in other breeds; so that the condition of dominance found in their original stock is to be regarded as a human artifact produced by the selection of breeders for the complete expression of rumplessness. Since the complete expression of rumplessness induces much sterility, this selection must have been chiefly exerted on heterozygotes. In selecting for Mild expression Dunn and Landauer were merely undoing the work by which the completely rumpless heterozygote had been produced. It is, therefore, unnecessary to postulate, as Dunn and Landauer do, that the modifiers they have selected "would be retained in the normal type because of their obviously favourable effect on development". On the contrary, they admit that "No apparent effect of these genes on the normal type has been observed". The favourable effect on development seems only to occur in the presence of the gene for rumplessness; the simplest view is that they have been established in the wild species in response to the continual recurrence of that gene as a mutant over a very long period.

4. THE SUPPOSITION THAT DOMINANCE HAS BEEN MODIFIED BY SELECTION OF THE NON-MUTANT

Hutchinson's results with Crinkled Dwarf

A second case in which dominance modification has been demonstrated, but in which it has been thought that the modification should be ascribed to the selective action of modifiers in the non-mutant homozygote, is one to which I alluded in 1930 on the basis of information supplied by Mr J. B. Hutchinson ("Genetical Theory of Natural Selection", p. 59):

> An extremely interesting case showing the modification of the heterozygote so far as to be indistinguishable from the non-mutant, that is of the acquisition of complete dominance by the wild type gene, has been brought to my notice by Mr J. B. Hutchinson from the work of Dr C. S. Harland on the genetics of the cotton plant. The several species of new-world cottons can be freely intercrossed and yield fertile offspring. One of these, the Sea Island cotton, has repeatedly produced a mutant form known as Crinkled Dwarf, which in that species is completely recessive. It appears to be identical with a similar mutant known as Wrinkled Leaf, appearing in some nearly related forms grown in Egypt, but so far as is known none of the other American species throw this mutant. In the course of Dr Harland's experiments the Crinkled Dwarf mutation of Sea Island was crossed with two other new-world species, Upland and Peruvian. The outstanding results of the cross were the same in both cases. The heterozygote was found to be slightly affected by the mutant character, thus indicating, even at this stage, some incompleteness of dominance. The most remarkable effects, however, were produced in the second generation, derived from the heterozygote by self fertilization. In this we should expect a quarter of the offspring to be Crinkled Dwarf, a half to be heterozygote, and a quarter to be non-mutant. The homozygous forms appeared as expected, but were connected by a practically continuous series of intermediate types. The heterozygotes in fact showed dominance of all grades. It is evident that the Sea Island cotton differed from the other new-world species in a number of modifying factors affecting the development and appearance of the heterozygote, the combined effect of which, in the Sea Island species, is to render the heterozygote normal in appearance. In this case the complete modification in the reaction of the organism to the mutant gene must have been brought about since the separation of this species from its new-world congeners; the whole process of evolution from the first appearance of the mutation, at least with appreciable frequency, must therefore have been comparatively rapid. When the mutation rate has been determined this case should afford a useful guide to the extent of the analogous events which we should expect to have taken place in other species.

Harland later showed that in two cases repeated back-crosses to Upland strains, namely *Triumph* and *Virescent Yellow*, resulted in the re-establishment of dominance. Since Harland believed that the mutations did not spontaneously occur in the Upland cottons (*Gossypium hirsutum*), the fact that this species contained a "modifier complex" producing complete dominance led him to suppose that these modifiers must have been selected for their effects on the non-mutant homozygote. Such effects are, indeed, hypothetical, but would seem to be a fair inference from the facts when complete absence of this mutation from the Upland species is postulated. In 1933, however, Hutchinson discovered a mutation occurring spontaneously in selections of Upland cotton grown at Indore, and later proved that this Indore Crinkled was identical with the Crinkled Dwarf previously discovered, as well as with the Wrinkled Leaf known in Egypt.

It thus appears that both the Sea Island cotton (*G. barbadense*) and the Upland cottons (*G. hirsutum*) are subject to this mutation, and that, in both, the mutation has become completely recessive. In *G. hirsutum*, indeed, Hutchinson shows that the homozygous mutant has been modified somewhat further towards normality than in *G. barbadense*. The modifiers by which dominance is produced in the two species are, however, largely different. Hutchinson therefore concludes that the occurrence of the mutation antedates the separation of the two species, but that, owing to the low initial viability of the heterozygote, the development of dominance was for long extremely slow, becoming progressively more rapid as the differences in viability between heterozygote and normal decreased. He suggests further that the rate of improvement must have been accelerated in recent times by changes in the method of cultivation. The cultivation of the primitive perennial forms of *G. barbadense* by the South American Indians was very crude, and reproduction was usually from a cluster of seeds. With intense competition among seedlings the survival of the Crinkled heterozygote would be very rare, and its rate of improvement correspondingly slow. Under modern cultivation, with a low seed rate and early thinning of seedlings, there is a much greater chance of a slightly Crinkled heterozygote yielding seed. In addition, the development of the annual habit must have increased the rate of improvement by allowing four or five generations in the period formerly occupied by one. Since Upland cottons have longer been cultivated as annual types, modification towards normality is more advanced in this species.

Hutchinson concludes that all the available evidence agrees strikingly with expectation on the selection theory, and, while not inconsistent with Harland's theory of selection of modifiers on their own account, provides no support for it.

5. ROSE COMB

The case of Rose comb is one of the most interesting of those found among the seven factors studied. The breeding data summarized in Table III are, indeed, inconclusive, since the one homozygote, proved to be such by breeding test, did not appear to differ in any clear characteristic from his heterozygous brethren. Nevertheless, the chance observation, made late in the experiment, that the gene for Rose comb also produces very noticeable changes in the bones of the head, will, it may be anticipated, enable workers with domesticated breeds to determine the degree of dominance exhibited in this case with a metrical precision far beyond what could be obtained from such scanty numbers as I have been able to breed.

Apart from this effect on the skull, Rose comb would be a most difficult structural feature in which to study dominance, especially in the wild jungle fowl. In the adult male the comb is much depressed vertically, though expanded laterally. The expanded upper surface presents a complicated pattern of ridges and tubercles, showing no constancy from bird to bird. The posterior end may be single, though not prolonged as in most of the fancy breeds, but rather shaped like a rudder, or it may be trifid, as it was with the one male proved to be homozygous. Whereas, with the adult males, it is the complex inconstancy of the organ which makes classification difficult, in the females and in immature birds of both sexes, the Rose comb is so slightly developed that variations in its structure would be likely to be imperceptible without a minute examination. Although the character can be diagnosed at hatching by the absence of the lamina of a single comb, the lack of development in young birds and females has reduced the material available for the study of the range of normal variability among heterozygotes to numbers really trifling in comparison with those of the other characters studied in this experiment.

It was in the preparation of heads for the examination of the hernia, found to accompany homozygosis in Crested birds, that it was noticed that birds with Rose comb had not the comb only widened, but also the frontal bone between the orbits. The extent of this widening may be gauged by measurements on the prepared skull, and, in the case of adult males, the width is increased, on the average, from 12 to 15 mm., or by about 25%. The lateral distance between the outer branches of the nasal bones is also materially increased, but the intra-orbital width of the frontal bone affords an entirely convenient measurement for metrical study. This measurement also is accessible on the living bird. The completeness or incompleteness of dominance in the case of Rose comb may therefore be satisfactorily determined from the average measurements of suitably large samples of the three genotypes, Single comb, heterozygous Rose, and homozygous Rose. Since many strains of popular breeds, such as the Rhode Island Red, have both single and rose combs, such material should be available to many workers in poultry genetics without special breeding experiments. I understand that Dr A. E. Brandt, of the State College of Agriculture of Ames, Iowa, has already material of this kind in view, and its examination will, I hope, establish the status of the Rose-comb factor as a complete or partial dominant far more decisively than could be hoped from any discussion of variations in the external structure.

The examination of domesticated material should not, in my opinion, be in any way inferior to that of the wild jungle fowl for such a metrical study. Whereas fanciers have been infinitely solicitous with regard to the external form of the comb, the simultaneous widening of the frontal bone seems to have escaped the notice even of poultry geneticists, and there appear to be no strong grounds for fearing that dominance in this character has been appreciably modified by human selection.

It is a matter of some general interest that, of the four structural characters tested in this experiment, all, without exception, should affect the development of the skeleton. In my previous communication it was shown, as further breeding has confirmed, that the gene responsible for Crest causes, when homozygous, an opening of the frontal bone leading to cerebral hernia. In the present paper it has appeared that the gene responsible for Feathered feet caused, when homozygous, a pronounced brachydactyly, with the suppression of the terminal phalanx of the outermost toe. This is also partially brought about, in some genic combinations, in the heterozygote. The fourth structural character studied has been polydactyly, the skeletal nature of which has never been in doubt. In view of these examples the remarkable conservatism of the skeleton in phylogeny should certainly not be ascribed to any lack of abundance of mutations affecting its structure, but rather to the persistent success of selection in preventing such mutations, as must constantly be occurring, from having any evolutionary effect, save in favourable and most exceptional circumstances.

TABLE III. MATINGS INVOLVING ROSE COMB

	Back-crosses	Normal	Rose	Total
1929	Purchased Silkies by Wild ♂	19	15	34
1930	9 Rose (1929) ♀♀ × Wild ♂♂	65	78	143
1931	Mixed pens of 1930 ♀♀ × Wild ♂♂; no segregation obtainable			
1932	Wild ♀♀ × (1931) Rose ♂	2	8	10
1933	4 Rose ♀♀ × Wild ♂	4	4	8
	Total of four generations back-cross	90	105	195
	Intercrosses			
1934	2 Rose (1933) ♀♀ × Rose (1933) ♂	6	11	17
1935	Idem adding 1 (1934) ♀	5	9	14
		11	20	31
	Test matings			
1935	Single ♀♀ × Rose ♂ (1934A)	—	11	11
1936	Idem	—	9	9
		—	20	20
1935	Single ♀♀ × Rose ♂ (1934B)	3	7	10
1935	Single ♀♀ × Rose ♂ (1934C)	3	7	10

The first matings (1929) shows that the Silky hens were probably all heterozygous for Rose. The ratio 90 : 105 does not differ significantly from unity.

If the 1934 female bred in 1935 she was apparently heterozygous, since there is an excess of normals from the intercross matings in both years; the numbers differ widely from the 1 : 5 expectation, but not greatly from an expectation of 1 : 3.

Of the three males from 1934 successfully tested, one is clearly homozygous, and two heterozygous. Of the two other Rose males from this year one died in 1935 without breeding, while the other failed to breed both in 1935 and 1936. This was, unfortunately, the only other male of this year with a trifid comb.

6. BLACK INTERNAL PIGMENT

The factor for black internal pigment produces an intense black pigmentation on many internal membranes. It was doubtless this factor which led Darwin to describe the Japanese Silky as the "black-boned Silk fowl". Externally, it may be easily recognized at hatching by the feet being a deep green instead of yellow. Throughout life the comb and wattles and bare skin of the face are usually somewhat darkened, the effect being more striking and regular on the underside of the wing. It has been propounded that the pigmentation caused by this factor is characteristically mesodermal, and, if it is exclusively so, the peculiar down plumage, mentioned below, must be ascribed to some other factor. For, although it will be shown that in this factor, as in others, dominance is irregular, and controlled in its degree by other genetic factors, the few birds which it has been possible to breed in this line do not suffice to demonstrate that the characteristic down plumage observed is really a manifestation of homozygosis.

Of the thirty-four chicks bred from the first cross (1929) of Silky hens with a wild *Gallus* cock, thirty-two were pigmented and two unpigmented. This aberrant ratio might perhaps be due to one of the four bought hens being heterozygous and the remainder homozygous for the mutant gene. However this may be, manifestation of the pigmentation was notably variable in this first cross, and it was thought at the time that the factor might have to be abandoned as too difficult for the study of dominance. The variability, however, diminished greatly in later generations.

Nine pigmented hens, bred to wild cocks in 1930, gave a regular segregation of seventy-eight pigmented to sixty-six unpigmented. Pigmented birds were bred in two pens along with other factors in 1931, and two pigmented females were available for a fourth cross to the wild in 1932. These, constituting now a separate line, gave ten chicks, six of which were pigmented, while only two, a male and a female, survived for breeding.

In order to follow the original plan for making five crosses to wild it would have been better at this point to have mated the cock to wild hens. The wild line had, however, bred so badly that likely pullets were not available, and, rather than abandon the factor, the two surviving birds were intercrossed in 1933. They gave six pigmented to one normal, of which again a pigmented pair survived. The parental cross could not, unfortunately, be repeated, owing to the death of the female. The male used in 1934 was proved later to be homozygous, and it is a most unfortunate omission, in view of his progeny, that his down characters were not noted.

Of the ten chicks, hatched in 1934, one, a female, showed much additional pigmentation in the down of the head. The normal eye to ear stripes were extended backward (see fig. 1) to meet the median band at the back of the head, and there were additional spots above and in front of the eyes. This extension of the dark brown pigment seemed associated, in many of the chicks that showed it in later years, with a lightening of the area to a less dark brown. The probability that it is an effect of the factor for internal pigment, rather than that it is due to some other factor happening to segregate when the line was inbred, is suggested by the observation, which was carefully confirmed in later years, that the feet, at about a week, were

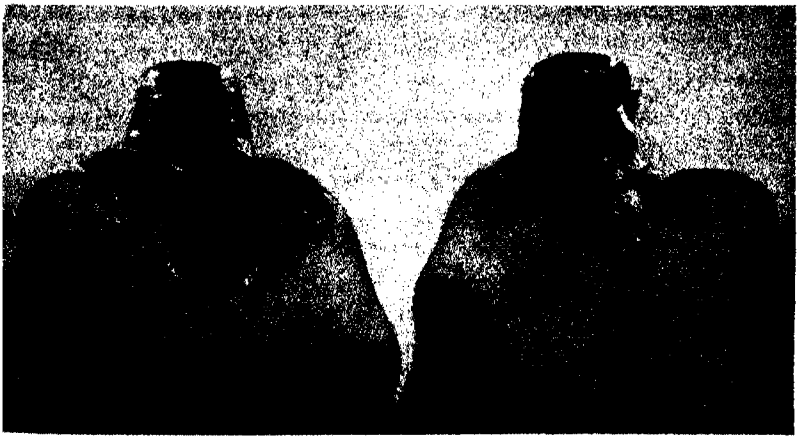

FIG. 1. Photographs of chickens showing: right, abnormal extension of brown pigment on the sides and back of the head; left, chick from the same brood classified as normal.

so much darkened as to appear black rather than dark green. This observation, combined with the fact that the new down pattern only appeared in this line, and in broods which should have contained some homozygotes, give probability to the view that black internal pigment, like Crest, Barred, Polydactyly and Feathered feet, has a special homozygous manifestation. The experiments yield no evidence contrary to this view, but are insufficient to establish it with confidence. The numbers showing normal and extended down plumage have regularly departed from the 1 : 1 ratio which, in the absence of genetic complications, would be expected.

In 1935 the same male, mated with the same hen as before, together with two 1934 daughters having normal down, bred thirty-three chicks, of which eleven had extended pigmentation in the down. In 1936 the same pen gave two with extended pigmentation out of five. On the total of 3 years, only fourteen out of forty-eight showed the down character, a proportion more nearly a quarter than a half.

The attempt to test the abnormal female of 1934 by outcrossing failed in both of the following years. One female with abnormal down from 1935 was successfully outcrossed in 1936, and gave five pigmented young. She was, therefore, probably homozygous, and this test, so far as it goes, supports the view that the extended down pigmentation is an indication of homozygosity.

It may be noted that the homozygous male possessed, when adult, a brown, instead of a black breast, and that the adult plumage of the two females reared from abnormally marked chicks both differed from wild females in the lighter buffish tone of their plumage.

7. VARIABLE DOMINANCE SHOWN BY EARLY EXPERIMENTS WITH BLACK INTERNAL PIGMENT

Proof that the dominance of the factor for Black Internal pigment, whatever its status in the wild fowl, is subject to modification in breed crosses, is afforded by an extensive experiment reported by Bateson and Punnett (1911).

Reciprocal crosses between Silky fowls and Brown Leghorns showed that the latter breed contained a factor, referred to as an inhibitor (**I**), which reduced the visible pigmentation due to the factor from the Silky. This inhibitor was sex-linked.

Although the experiment was prolonged, and many different crosses were made, it was not carried out in such a way as to exhibit the manifestation in all the genotypes, six in number for females, and nine for males, produced by combining an autosomal with a sex-linked factor. Most of the broods were classified in three classes, deeply pigmented, slightly pigmented, and unpigmented. It is, however, not clear whether all parts of the body, or perhaps only the feet were referred to in this classification.

The fifteen genotypes are shown in the following plan, in which **G** is used for the gene for black internal pigment, and **I** for the inhibitor.

	Females		Males		
	I–	**i–**	**II**	**Ii**	**ii**
GG	—	Deep	—	—	Deep
Gg	Slight	Usually deep	Slight	Slight	Deep
gg	Unpigmented	Unpigmented	Unpigmented	Unpigmented	Unpigmented

The whole of the data are consistent with the following statements:

(*a*) All **gg** genotypes are unpigmented.

(*b*) **GGi–** females, and **GGii** males are deeply pigmented.

(*c*) **GgI–** females and **GgII** males are slightly pigmented.

(*d*) **GgIi** males are slightly pigmented, with one exception classified as deeply pigmented out of more than 100 (Table XI).

(*e*) **Ggi–** females are nearly always deeply pigmented, but nearly 4% are classified as slightly pigmented.

(*f*) **Ggii** males are apparently always deeply pigmented.

The evidence of the data bearing on statement (*e*) may be summarized by comparing the numbers of deeply and slightly pigmented females with the numbers expected, on the supposition that 3·9% of the genotype **Ggi–** were classified as slightly pigmented.

TABLE IV. BROODS SHOWING THE CLASSIFICATION OF **Ggi–** FEMALES

	Deeply pigmented		
Reference	Expected	Observed	Total females
Table II	38·44	39	40
V	86·97	82	362
VII	58·83	56	60
VIII IX	59·81	61	61
X	10·09	9	21
XI	100·90	101	105
XII (*a*)	2·88	3	6
XII (*b*)	24·99	26	26
Total	382·91	377	681

The agreement with expectation is seen to be excellent throughout. The forty birds of Table II, the 105 of Table XI and the twenty-six of Table XII (*b*) were all of genotype **GgIi**. From these alone an estimate of five cases out of 171, or 2·92 %, slightly pigmented could have been made. The sixty birds of Table VII should have been **GGi–** and **Ggi–** in equal numbers; the four slightly pigmented were presumably all **Ggi–** birds. The similar matings of Tables VIII and IX gave no exceptions. The three types of mating of Tables V, X and XII (*a*) all show genotypes **Ggi–** mixed with other genotypes showing slight or no pigmentation; they all agree with expectation when most of the **GGi–** birds are expected to be deeply pigmented.

The consistent results for the heterozygote in the absence of the inhibitor (**Ggi–**) enable us to investigate the homozygote in the presence of the inhibitor (**GGI–**), for which only indirect data are available. These show some discrepancy, though not to the extreme extent shown by the males.

Table III shows seventy-four deeply to fifty-five slightly pigmented from a mating **GgI–** × **GgIi** which should give among the pigmented birds

2GgI– + 2Ggi– + 1GGI– + 1GGi–.

We may estimate the behaviour of the homozygotes containing the inhibitor by differences (Table V):

TABLE V. ALLOCATION OF **GGI–** FEMALES IN TABLE III

	Deeply	Slightly
GgI–	—	43·00
Ggi–	41·32	1·68
GGi–	21·50	—
GGI–	11·18	10·32
Observed total	74	55

Apparently more than half the homozygotes containing the inhibitor show full pigmentation.

The reciprocal cross has the same expectation in the females (Table VI).

TABLE VI. ALLOCATION OF **GGI–** FEMALES IN TABLE IV

	Deeply	Slightly
GgI–	—	18·67
Ggi–	17·94	0·73
GGi–	9·33	—
GGI–	0·73	8·60
Observed total	28	28

In this case the homozygotes appear to be almost completely inhibited.

The mating of Table VI should give the same four genotypes now in equal numbers; there are, however, only forty-nine females classified.

TABLE VII. ALLOCATION OF **GGI–** FEMALES IN TABLE VI

	Deeply	Slightly
GgI–	—	12·25
Ggi–	11·77	0·48
GGi–	12·25	—
GGI–	−2·02	14·27
Observed total	22	27

There is again in this experiment no evidence of the homozygotes being deeply pigmented. The view that no appreciable fraction show deep pigmentation is, however, scarcely compatible with the data of Table III, for which it would give:

TABLE VIII. TEST OF SIGNIFICANCE FOR TABLE III

	Expected (m)	Observed (a)	$a-m$	$\frac{(a-m)^2}{m}$
Deeply	62·82	74	11·18	1·990
Slightly	66·18	55	−11·18	1·889
Total	129	129		3·879

The value of 3·879 exceeds its 5% value for one degree of freedom, so that it is probable, though not certain, that even in the female an appreciable fraction of homozygotes carrying the inhibitor were yet classified as deeply pigmented. Since deep pigmentation does not occur among the heterozygotes **GgI–**, this would show that in the presence of **I** the factor **G** was not completely dominant. If the whole of the data for females is used to estimate the proportion of those homozygotes deeply pigmented it appears that this is most probably as high as 17·5%. With this value the expectations are as follows:

TABLE IX. TEST OF HETEROGENEITY OF DIFFERENT TABLES

		Expected	Observed	Deviation
Table III:	Deeply	66·59	74	−7·41
	Slightly	62·41	55	+7·41
Table IV:	Deeply	28·91	28	+0·91
	Slightly	27·09	28	−0·91
Table VI:	Deeply	26·17	22	+4·17
	Slightly	22·83	27	−4·17

The observed values do not differ significantly from expectation. $\chi^2 = 3 \cdot 188$ for 2 degrees of freedom is exceeded by chance more than once in five times. It is a possible view that the proportion deeply pigmented was uniformly at about $17\frac{1}{2}\%$. More probably perhaps it was higher in the larger experiment, and negligible in the two smaller ones.

The evidence for the males is more decisive. We will first show that the heterozygotes without the inhibitor (**Ggii**) were uniformly classified as deeply pigmented. The relevant types of mating were as follows:

TABLE X. BROODS SHOWING CLASSIFICATION OF **Ggii** MALES

	Deeply pigmented		Total birds
	Expected	Observed	
Table VIII	42	42	42
IX	21	21	21
X	14·5	14	29
XII (*a*)	2·5	1	5
XII (*b*)	18	18	18
Total	98	96	115

So far as the published classification of pigmentation goes, the factor for black internal pigment was completely dominant in the absence of the inhibitor.

With the inhibitor heterozygous (**Ii**) we must consider the classification of the homozygotes (**GGIi**). Table VI gives the classification of the mating **GGi**– × **GgIi** yielding the four genotypes

GGii, **GGIi**, **Ggii** and **GgIi**,

in equal numbers; if ν is the proportion deeply pigmented among the homozygotes (**GGIi**) the expectations are $2+\nu$ deeply to $2-\nu$ slightly pigmented. The observed frequencies are 25 : 20, giving the estimate $22 \cdot 2\%$ for ν.

In contrast, Table VII gives the classification for the mating **GgI**– × **GGii** yielding the genotypes

GGIi and **GgIi**.

The expectations are $\nu : 2-\nu$ which with the observed frequencies 24 : 31 gives an estimate $\nu = 87 \cdot 3\%$.

The pigmented birds from Table III **Ggi**–♀♀ × **GgIi**♂♂ should be of the genotypes

2GgIi + 2Ggii + GGIi + GGii

giving an expected ratio $3+\nu : 3-\nu$ with observed frequencies 51 : 55. Here clearly ν is zero or negligible.

Finally, Table IV gives the result of the mating **GgI–**♀♀ × **GgIi**♂♂, of which the pigmented young should be

$$2\mathbf{GgII} + 2\mathbf{GgIi} + \mathbf{GGII} + \mathbf{GGIi}.$$

We may assume that **II** genotypes are not more strongly pigmented than the corresponding **Ii** genotype. If in the homozygotes the inhibitor was completely recessive the expectation is still only $\nu : 3-\nu$. With observed values 12 : 42 this gives the estimate $\nu = 66\cdot7$ %; while if it is supposed that **II** birds are never deeply pigmented, the expectation is $\nu : 6-\nu$ which with $\nu = 100$ %, is only 9 : 45 against 12 : 42 observed.

It is evident that the proportion of homozygotes heterozygous for the inhibitor varies in these experiments nearly from 0 to 100 %; i.e. that **G** varies from a dominant to an almost complete recessive. For thoroughness I have worked out the best fitting frequency (*a*) on the supposition that **II** birds are never deeply pigmented, and also (*b*) on the supposition that they are as frequently deeply pigmented as are **Ii** birds. In both cases it appears that an appreciable fraction of **GG** birds must be deeply pigmented, when the corresponding **Gg** birds are not so, so that the "inhibitor" itself acts powerfully as a dominance modifier; and that the data are significantly heterogeneous in respect of the degree of dominance shown in different matings. Probably many other factors affecting dominance were segregating in the second inbred generation.

TABLE XI. DISCREPANCIES AND AMOUNTS OF INFORMATION FROM DIFFERENT EXPERIMENTS

GGII ♂♂ 72·82 deeply pigmented.			**GGII** ♂♂ all slightly pigmented.		
Table VI	$25/(2+\nu)$	9·16355	Table VII	$24/\nu$	32·95798
	$20/(2-\nu)$	15·72574		$31/(2-\nu)$	24·37490
Discrepancy		−6·56219	Discrepancy		+8·58308
Information	$45/(4-\nu^2)$	12·969	Information	$55/\nu(2-\nu)$	59·387
Table III	$51/(3+\nu)$	13·67952	Table IV	$12/\nu$	16·47899
	$55/(3-\nu)$	24·20988		$42/(6-\nu)$	7·96692
Discrepancy		−10·53036	Discrepancy		+8·51207
Information	$106/(9-\nu^2)$	12·515	Information	$54/\nu(6-\nu)$	14·066

Summary of four experiments ($\nu = 72\cdot82$ %)

	Discrepancy D	Information I	D^2/I
Table VI	−6·56219	12·969	3·3204
Table VII	+8·58308	59·387	1·2405
Table III	−10·53036	12·515	8·8604
Table IV	+8·51207	14·066	5·1511
Total	+0·00260	98·937	−0·0000
$\chi^2(n=3)$			18·5724

TABLE XII. DISCREPANCIES AND AMOUNTS OF INFORMATION FROM DIFFERENT EXPERIMENTS

GGIi ♂♂ and **GGII** ♂♂ both 64·81 deeply pigmented.

Table VI	$25/(2+\nu)$	9·44073	Table VII	$24/\nu$	37·03132
	$20/(2-\nu)$	14·79399		$31/(2-\nu)$	22·93069
Discrepancy		−5·35326	Discrepancy		+14·10063
Information	$45/(4-\nu^2)$	12·570	Information	$55/\nu(2-\nu)$	62·630
Table III	$51/(3+\nu)$	13·97988	Table IV	$12/\nu$	18·51566
	$55/(3-\nu)$	23·38535		$42/(3-\nu)$	17·85790
Discrepancy		−9·40547	Discrepancy		+0·65776
Information	$106/(9-\nu^2)$	12·354	Information	$54/\nu(3-\nu)$	35·427

Summary of four experiments ($\nu = 64{\cdot}81\,\%$)

	Discrepancy D	Information I	D^2/I
Table VI	−5·35326	12·570	2·2798
Table VII	+14·10063	62·630	3·1746
Table III	−9·40547	12·354	7·1607
Table IV	+0·65776	35·427	0·0122
Total	−0·00034	122·981	−0·0000
$\chi^2(n=3)$			12·6273

For 3 degrees of freedom the 1 % value of χ^2 is only 11·341, so that on both hypotheses heterogeneity in dominance is strongly significant.

On the one extreme view the proportion of homozygotes (heterozygous for the inhibitor) classified as deeply pigmented is 72·82 %. Since scarcely any double heterozygotes are so classified the factor **G** is certainly not here acting as a dominant. Moreover the heterogeneity of the different sets ($\chi^2 = 18{\cdot}57$, for 3 degrees of freedom) shows that the degree of dominance varies very much in the different cases observed. At the other extreme, when **GGII** and **GGIi** birds are supposed to be equally frequently deeply pigmented, the percentage for those two genotypes comes to 64·81 %, with, in this case, somewhat less evidence of heterogeneity $\chi^2 = 12{\cdot}63$. On any view, therefore, the evidence of the males is decisive in showing that dominance of the factor for black internal pigment is greatly affected by the "inhibitor", and probably much affected also by other factors segregating in the same cross.

8. PILE (DOMINANT WHITE)

The factor commonly known in the literature of poultry genetics as "Dominant White", I propose to refer to as Pile (**Pi**), since it is not a dominant. Moreover, when acting alone on the wild constitution, it does not produce a completely white bird; but rather the pattern known by fanciers as "Pile", in which, though the large feathers are white, the wings of the males are a deep chestnut, while yellow and brown coloration is distributed on the heads and necks, and especially the breasts of females.

Some difficulty was encountered in introducing Pile into wild stock, since all the domestic birds available carrying this factor were somewhat large. However, in 1930, broods were obtained by reciprocal crosses using a small White Leghorn female, and a male of the same breed which was sexually mature at less than six months old. As has often been observed in Leghorn crosses the young were not wholly white, but irregularly suffused with yellow, and marked with black spots. One pen of pullets from this cross mated in 1931 to a wild cock gave twenty-one Pile to fifteen normal, counting as normal all young without the Pile factor, irrespective of the black and barred, which were also segregating in this cross. Seven more Pile young were bred in this year from a mixed pen in which both white and coloured mothers were used.

In 1932 again eight Pile females were crossed with a wild male. Although many eggs were laid only twenty-eight chicks were hatched, fifteen Pile to thirteen normal, and of these only two Pile females survived to lay in 1933. These gave a single brood of six Pile to three normal. Of these one Pile male and three Pile females were available for interbreeding in 1934. In this factor, therefore, as with Feathered feet and Black Internal pigment, the intercross was made after only four generations crossing with wild.

The intercross mating gave, in five broods, twenty-one chickens, of which only three lacked the Pile factor. Of the eighteen Piles, however, only two males and two females attained adult plumage. Of these two, one male and one female attracted attention by having less yellow on the head and neck than was usual, and were judged to be possibly homozygous. In the following year the male was mated with normal females, giving a brood of six Pile chicks. Unfortunately, he died before the chicks were hatched, and before it was realized that homozygotes lack the small coloured specks on the large feathers of the wings and tail, found regularly in heterozygotes. In consequence this important indication was not noted. His brother proved to be heterozygous by a similar test mating, and in this case the feathers were speckled in the usual manner.

The significance of these small spots on the feathers was first noted by A. E. Brandt, who was kindly assisting the author in 1935. They do not appear on the first true plumage, so that at this stage both heterozygotes and homozygotes have clean, or unspecked feathers. In the second plumage, however, heterozygotes invariably show speckling on almost every feather in the tail and wing primaries, while they are completely absent from homozygotes. Brandt has already published the evidence, based on the examination of numerous purebred and crossbred White Leghorns, on which this conclusion was based.

The interbreeding was repeated in 1935 with the addition of two 1934 pullets to the original pen, but only gave eleven Pile and four normal young. Although one of the five hens in the pen this year was a homozygote it is probable that she did not breed, as the attempt to breed from her in 1936 failed completely. A third homozygote did appear, however, among the 1935 birds, though she also failed to breed in 1936. Had these two years been favourable, as judged by success in other pens, the failure of these two hens to breed would give a presumption that homozygosity in this factor was associated with infertility. In reality, however, all the strains having a high proportion of wild blood were extremely disappointing in these two years, and many other individual birds, besides these two, failed to provide the test broods.

The most definite diagnostic of homozygosity in this factor is undoubtedly supplied by the small pigmented areas on the larger feathers. This indication is the more valuable as it is available in ordinary breed crosses, and will make linkage tests materially easier. In birds of nearly wild ancestry, also, the homozygote is distinguishable by the paler coloration of the head and neck, and, in the case of the females, by a very striking difference in the depth of pigment on the breast.* Pile is not, like barred and feathered feet, more nearly recessive than dominant, on the contrary the heterozygote differs more strikingly from the wild type than from the homozygote; but it differs so decidedly from the latter in adult birds that dominance must be regarded as definitely incomplete.

9. GENERAL SUMMARY

The experiments with wild *Gallus* have dealt with seven reputed dominants among the mutant genes known in the domestic fowl. They have shown that in six of these cases dominance is far from complete. The seventh case is that of Rose comb, for which the present data are inconclusive, although on the basis of the observations presented a very exact determination of the quantitative degree of dominance should soon be possible in domestic material. In some cases the incompleteness of dominance is clearly demonstrated from my own material alone, in others the demonstration receives material support from the data of others.

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* Apparently this difference also is sometimes observable in domesticated breeds, for Punnett, p. 142, observes that "Breeders of exhibition piles recommend the cross with the black-red to deepen the colour of the orange markings. Probably this is because the depth of the orange is affected in a bird which is homozygous".

Three of the factors concerned affect pigmentation, Barred plumage, Pile, and Black Internal pigment. In all these three dominance is incomplete; in the case of Bar the mutant gene is more nearly recessive than dominant. With Bar and Pile the homozygous manifestation is unmistakable. I have not been able to determine whether the peculiar down pattern observed is a homozygous manifestation of Black Internal pigment, but the data of Bateson and Punnett show that dominance in this factor is certainly much influenced by other genetic factors. The apparent dominance of Bar in domestic breeds appears to be largely due to its association with a factor for black. It is possible that the apparent dominance of Pile may be enhanced by association with Bar and Silver.

The four other factors are structural. These are Rose comb, Crest, Polydactyly and Feathered feet. It is remarkable that all these affect the skeleton. Rose comb widens the frontal bone between the orbits; it is not yet known whether this widening is equal in homozygous and in heterozygous birds, or greater in the homozygote. Rose comb may therefore be a true dominant. Crest, when homozygous, not only enlarges the tuft of feathers on the head, but produces an obvious cerebral hernia. Its apparent dominance in the Silky breed appears to be due to a suppression by modifying factors of this dangerous manifestation. In polydactyly the bones of the foot in wild stock are nearly intermediate in the heterozygote between their structures in the two homozygotes. In breed crosses dominance in this factor appears to be widely variable. In Feathered feet the homozygote differs in many respects from the heterogyzote, notably, in my material, in the brachydactyly or dwarfing of the fourth toe, and in the extent and nature of the feathering. Apparent dominance may have been produced in some breeds by genes capable of mitigating this deformity. In both Feathered feet and Polydactyly the heterozygote is often indistinguishable from the normal, as with complete recessives.

Regarding these factors as a group they appear to be quite analogous to the imperfect "dominant" mutations known as such in *Drosophila*. It must be remembered that they were chosen out of the material available in poultry as the best authenticated dominants known. Earlier work has shown the incompleteness of dominance in many other factors in poultry such as Pea comb, Frizzled plumage, Rumpless, Silver, and Spangled, in which dominance was at first postulated, and in one case, the Andalusian

Blue, in which absence of dominance was recognized from the first. An inevitable conclusion from the results presented is that the wild species from which domestic poultry are derived provides no exception to the genetical situation found in other organisms, in which, while completely recessive mutant genes are common, complete dominance of the mutant is absent or exceptional. The striking point in the genetics of fowls is the frequency of incomplete dominants as compared with complete recessives (a consequence presumably of the conditions on which domestication was initiated), and the frequency with which apparent dominance has been produced, or enhanced, by the introduction of additional factors into the domestic breeds.

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615.751 : 541.6

Antiplasmodial action and chemical constitution

Part I. Cinchona alkaloidal derivatives and allied substances

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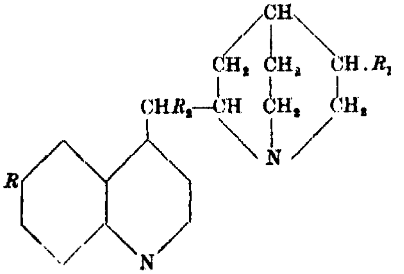
The constitution of the cinchona alkaloids is known with certainty, and some have been synthesized, but little is known as to why they are antiplasmodial in action. The introduction of experimental bird malaria has given a means of following changes in therapeutic activity with changes in chemical structure, and mainly through the work of Giemsa, Weise and Tropp (1926), Goodson, Henry and Macfie (1930), Schönhöfer (1933), Giemsa and Oesterlin (1933) and Buttle, Henry and Trevan (1934), some insight has been gained of the effect of simple changes of structure of the cinchona alkaloids on their antiplasmodial action in bird malaria. The present communication, in extension of the work of these observers, is designed primarily to furnish additional data which might provide a working hypothesis for further experiments.

The chief naturally occurring cinchona alkaloids are embraced by the formula (I):



(I)

where R may be H or OMe, R_1 may be $.CH{:}CH_2$ or $.CH_2.CH_3$ and R_2 is OH, and so far as the results of their therapeutic examination on bird malaria due to *Plasmodium praecox* or *inconstans* have been recorded by previous workers they are all active. We have prepared seven chloro-alkaloids (I, $R_2 = Cl$) by the action of phosphorus pentachloride on the hydrochloride of the bases (Comstock and Königs 1884) and, of these chloro-derivatives, ***hydrocinchonidine chloride*** has not been previously described. The following table shows that on the doses tried they were all inactive when tested orally on *Pl. relictum* (= *Pl. praecox*) in canaries, and this result amplifies a similar observation of Giemsa, Weise and Tropp (1926) on chloroquinine.

TABLE I

Substance	Dose in mg. per 20 g. of body weight	Day of appearance of parasites in blood	Remarks
Quinine chloride HCl	6 × 1·25*	6	M.T.D.†
Hydroquinine chloride HCl	6 × 1·25	5	M.T.D.
Quinidine chloride HCl	6 × 0·625	5	M.T.D.
Hydroquinidine chloride HCl	6 × 1·25	5	M.T.D.
Cinchonine chloride HCl	6 × 1·25	5	M.T.D.
Cinchonidine chloride HCl	6 × 1·25	5	M.T.D.
Hydrocinchonidine chloride HCl	1 × 2·5 + 5 × 1·25	7	M.T.D.
Quinine HCl	6 × 5	20	M.T.D.
No. of control birds, 9	—	5–6	—

* This means a dose of 1·25 mg. was given daily for 6 days, the first dose being administered 4 hr. after inoculation with malaria.

† M.T.D. = maximum tolerated dose.

When quinine chloride, cinchonine chloride and hydroquinine chloride were treated with alcoholic potash (Comstock and Königs 1884), quinene, cinchene and hydroquinene were formed, the bridge in the above formula becoming $-\mathrm{CH}{=}\mathrm{C}\langle$. The isolation of these bases was greatly facilitated by the use of their cuprichlorides (Cohen 1933). They, also, proved to be inactive on bird malaria (see Table II).

TABLE II

Substance	Dose in mg. per 20 g. of body weight	Day of appearance of parasites in blood	Remarks
Quinene HCl	6 × 1·25	6	M.T.D.
Hydroquinene 2HCl	5 × 2·5 + 1 × 1·25	5	M.T.D.
Cinchene 2HCl	6 × 1·25	6	M.T.D.
No. of control birds, 6	—	5–6	—

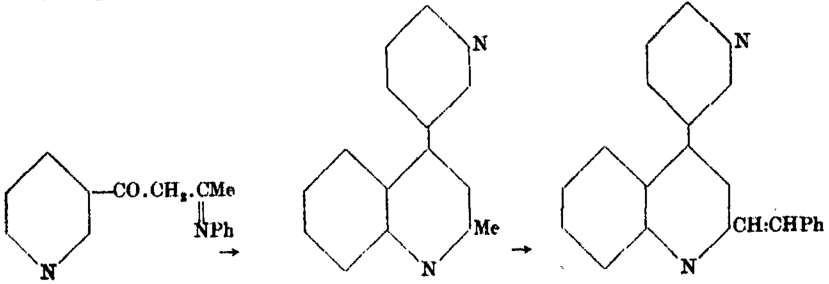
Desoxycinchonine and desoxycinchonidine (I, $R = \mathrm{H}$, $R_1 = .\mathrm{CH{:}CH_2}$, $R_2 = \mathrm{H}$) were prepared by the action of iron filings and dilute sulphuric acid on the corresponding chloro-alkaloids by the process of Königs (1895), but desoxydihydrocinchonine and desoxydihydrocinchonidine have been prepared by the catalytic reduction of cinchonine chloride and cinchonidine chloride respectively by hydrogen and palladized charcoal. This involves removal of the chlorine atom and replacement by hydrogen and also reduction of the vinyl group. Experiments interrupted before reduction was complete showed that hydrogenation of the vinyl group and replacement of the chlorine atom by hydrogen were proceeding simultaneously. It is not possible therefore by this means to prepare the desoxy-bases of the alkaloids containing vinyl groups, but the method is very suitable for the removal of chlorine from the chlorides of the dihydro-bases. These four desoxy-bases proved to be inactive on bird malaria, Table III.

TABLE III

Substance	Dose in mg. per 20 g. of body weight	Day of appearance of parasites in blood	Remarks
Desoxycinchonine HCl	6 × 1·25	5	M.T.D.
Desoxycinchonidine HCl	6 × 0·625	5	M.T.D.
Desoxydihydrocinchonine HCl	6 × 0·625	5	M.T.D.
Desoxydihydrocinchonidine HCl	6 × 0·625	5	M.T.D.
No. of control birds, 5	—	5–6	—

When the vinyl group in quinine is oxidized to carboxyl, quitenine is formed, an acidic substance which is therapeutically inactive (Giemsa, Weise and Tropp 1926), but when the carboxyl group is esterified with simple aliphatic alcohols, weak therapeutic activity is restored (Goodson, Henry and Macfie 1930). We have prepared *methylquitenine dihydrochloride* and by oxidation of cinchonine and cinchonidine and subsequent esterification we have prepared the analogously constituted *methylcinchotenine* and *methylcinchotenidine dihydrochlorides* respectively. Methylquitenine showed slight activity, in agreement with the observation of Goodson, Henry and Macfie, but methylcinchotenine and methylcinchotenidine were inactive, although according to the above-named observers the ethyl ester showed slight activity. By the action of ammonia and methylamine on methylquitenine, *quitenamide* and *quitenmethylamide* were respectively formed, but they showed no therapeutic action, Table IV.

Incidental to the above work, which is confined to experiments starting with cinchona alkaloids, a few synthetic experiments were instituted with β-pyridoylacetone which was available. When heated with aniline it gave the anil, *γ-phenylimido-α-keto-α-(3-pyridyl) butane* (II), which on solution in sulphuric acid and heating at 100° gave 2-*methyl*-4-(3-*pyridyl*) *quinoline* (III) (compare Tscherne 1901).



(II) (III) (IV)

TABLE IV

Substance	Dose in mg. per 20 g. of body weight	Day of appearance of parasites in blood	Remarks
Methylquitenine 2HCl	6 × 10	12	—
	6 × 10	7	—
Methylcinchotenine 2HCl	3 × 10 + 3 × 2·5	5	M.T.D.
Methylcinchotenidine 2HCl	2 × 10 + 4 × 5	5	M.T.D.
Quitenamide	6 × 10	5	—
	6 × 2·5	7	—
	6 × 2·5 sub. cut.	5	—
Quitenmethylamide	6 × 10	5	—
2-Methyl-4-(3-pyridino) quinoline 2HCl	2 × 1·25 + 4 × 0·625	5	M.T.D.
2-Styryl-4-β-pyridino-quinoline 2HCl	6 × 10	5	M.T.D.
No. of control birds, 12	—	5–6	—

This substituted quinaldine has a reactive methyl group and on boiling with benzaldehyde gave 2-*styryl*-4-(3-*pyridyl*) *quinoline* (IV). 2-Methyl-4-(3-pyridyl) quinoline is of interest, in that apart from a general structural similarity to cinchona alkaloids the tertiary pyridino-nitrogen atom is separated from the quinoline nucleus by two carbon atoms just as is the tertiary nitrogen atom of the quinuclidine nucleus in cinchonine. Nevertheless, it proved to be therapeutically inactive on bird malaria, as was also the styryl derivative.

It has already been mentioned that so far as data are available all the cinchona alkaloids are more or less antiplasmodial. As there are considerable stereochemical differences between the various cinchona alkaloids (King and Palmer 1922) it follows that antiplasmodial action is not very sensitive to such stereochemical changes. It is however sensitive to changes in structure, and the most striking result observed is the apparent loss of activity when the central —CHOH— group is modified in any way. Thus its alteration by conversion into —CHCl—, $—CH_2—$ or —CH= leads in almost all cases to an increase in toxicity and loss of antiplasmodial action. It is possible that the —CHOH— group acts as an anchoring group on certain structures in the parasitic substance and thus takes part in the mechanism of destruction of the parasite.

If the —CHOH— group is kept intact activity may be retained with some change of structure. When the vinyl group is oxidized to carboxyl, quitenine is formed which is inactive. This is to be expected, since its distribution in the tissues will be quite different from that of a base, and this is confirmed by the fact that on esterification and restoration of basic properties activity reappears. This was strikingly brought out in the series of esters prepared by Goodson, Henry and Macfie (1930). It is however very surprising to find that conversion of the ester group into an amide group results in apparent loss of activity.

EXPERIMENTAL

The so-called cinchona alkaloidal "chlorides" were in each case prepared essentially by Comstock and Königs' method (1884). The starting material was in every case the purest alkaloid, as supplied by Messrs Howards and Sons, Ilford.

Quinine chloride was obtained in 63 % yield, m.p. 145–150°, and then crystallized from aqueous methyl alcohol in prisms, m.p .151°, $[\alpha]_D^{20} + 62{\cdot}0°$; $[\alpha]_{5461}^{20} + 69{\cdot}6°$ ($c = 1{\cdot}0$ in absolute ethyl alcohol), in agreement with the data of Giemsa and Halberkann (1921). The neutral *hydrochloride* prepared in methyl alcohol crystallized on adding dry ether in fine needles, m.p. 219° (decomp.), after darkening earlier. (Found: Cl′, 9·2; MeO, 7·8. $C_{20}H_{23}ON_2Cl$, HCl requires Cl′, 9·4; MeO, 8·2 %.) $[\alpha]_D^{20} + 9{\cdot}75°$; $[\alpha]_{5461}^{20} + 5{\cdot}65°$ ($c = 1{\cdot}0$ in water).

Cinchonine chloride was obtained crystalline as the neutral monohydrochloride from methyl alcohol and ether in fine woolly needles, m.p. 210° after sintering at 200°. $[\alpha]_D^{20} + 47{\cdot}8°$; $[\alpha]_{5461}^{20} + 58{\cdot}6°$ ($c = 1{\cdot}003$ in water), in agreement with Rabe's data (1910). (Found: Cl, 9·9, 10·0; Calc. Cl′ 10·2 %.) From the hydrochloride, the base m.p. 70–72° was obtained crystalline from aqueous alcohol.

The less soluble *cinchonine chloride hydrobromide* crystallizes from methyl alcohol in stout needles, m.p. 228°. (Found: Br′, 20·4. $C_{19}H_{21}N_2Cl$, HBr requires Br′, 20·3 %.) *Cinchonidine chloride* crystallized directly from ether and on recrystallization had m.p. 108–109°. The neutral monohydrochloride separated from aqueous solution in hexagonal plates containing one molecule of water of crystallization. (Found: Loss at 100°, 3·8. $C_{19}H_{21}N_2Cl$, HCl, H_2O requires H_2O, 4·9 %.) From a mixture of methyl alcohol and ether it separated in fine flat needles, m.p. 229° (decomp.). (Found: Cl′, 10·1; Calc. Cl′, 10·2 %.) $[\alpha]_D^{20} + 33{\cdot}8°$; $[\alpha]_{5461}^{20} + 38{\cdot}2°$ ($c = 1{\cdot}066$ in water). As Rabe (1910) gives $[\alpha]_D^{24} + 24{\cdot}16°$ in water the specimen was recrystallized and gave $[\alpha]_D^{20} + 29{\cdot}8°$; $[\alpha]_{5461}^{20} + 35{\cdot}0°$ ($c = 1{\cdot}018$ in water).

Quinidine chloride was crystallized as the neutral monohydrochloride from ethyl alcohol (twelve parts) in colourless needles, m.p. 212° (decomp.), after turning yellow above 200°. (Found: Cl′, 9·0; N, 7·1. Calc. Cl′, 9·3; N, 7·4 %.) $[\alpha]_D^{22{\cdot}5} + 48{\cdot}8°$; $[\alpha]_{5461}^{22{\cdot}5} + 59{\cdot}5°$ ($c = 1{\cdot}046$ in water). Heidelberger and Jacobs (1920) give m.p. 199–206°, $[\alpha]_D^{15}$ 47·7°.

Hydroquinine chloride crystallized directly from the ethereal extract in cream-coloured prisms, m.p. 139–141°. When recrystallized from ether it separated in rhombic prisms, m.p. 143°. The neutral monohydrochloride crystallized from water in narrow plates or leaflets, m.p. 233° (decomp.). (Found: Cl′, 9·3; Calc. Cl′, 9·3 %.) $[\alpha]_D^{19} - 2{\cdot}4°$; $[\alpha]_{5461}^{19} - 7{\cdot}8°$ ($c = 1{\cdot}028$ in water). These data are in agreement with those of Heidelberger and Jacobs (1920).

Hydroquinidine chloride obtained as a gum from the ethereal extract gradually crystallized on contact with ligroin. The neutral monohydrochloride from alcoholic solution crystallized in fine hair-like needles which required eleven parts of boiling ethyl alcohol for recrystallization, m.p. 209–211° (decomp.), after turning yellow at 208°. (Found: Cl′, 9·0; Calc. Cl′, 9·3 %.) $[\alpha]_D^{21} + 41{\cdot}7°$ ($c = 1{\cdot}128$ in water), in agreement with Heidelberger and Jacobs' data (1920).

Hydrocinchonidine chloride was obtained crystalline as the neutral *monohydrochloride* from anhydrous ethyl alcohol in fine leaflets. From methyl alcohol on addition of ether it separated in small leaflets or plates, m.p. 233–235° (decomp.). (Found: Cl′, 10·0; N, 8·0. $C_{19}H_{23}N_2Cl$, HCl requires Cl′, 10·1; N, 8·0 %.) $[\alpha]_D^{20} + 24{\cdot}0°$; $[\alpha]_{5461}^{20} + 21{\cdot}5°$ ($c = 0{\cdot}5$ in water).

Desoxyquinine was prepared by reduction of quinine chloride with iron and dilute sulphuric acid as described by Königs (1896) and isolated first of all as the picrate, as described by Heidelberger and Jacobs (1920). The base was recovered, treated with an equal weight of cupric chloride in concentrated hydrochloric acid and the solution chilled. *Desoxyquinine cuprichloride* crystallized out in orange-yellow boat-shaped leaflets, m.p. 178° (decomp.). (Found: Cl′, 27·2. $C_{20}H_{24}ON_2$, 2HCl, $CuCl_2$ requires Cl′, 27·5 %.) On removal of the copper as sulphide and basification of the filtrate, desoxyquinine separated and gradually crystallized.

Desoxycinchonine was obtained crystalline from the concentrated ether extract of the basified reduction liquor. It crystallized from ether in prisms, m.p. 88–90°, and finally from hexane with m.p. 91°. The neutral *monohydrochloride* crystallized from methyl alcohol on addition of dry ether in needles, m.p. 183–185°, after shrinking at 175°. (Found: Cl, 11·6. $C_{19}H_{22}N_2$, HCl requires Cl, 11·3 %.) $[\alpha]_D^{20} + 80{\cdot}8°$; $[\alpha]_{5461}^{20} + 95{\cdot}2°$ ($c = 1{\cdot}0$ in water).

Desoxycinchonidine was obtained from the ethereal extract of the basified reduction liquor and was isolated by conversion into the cuprichloride. From this the *hydrochloride*, m.p. 178–180° (to a yellow liquid), was obtained, by careful addition of dry ether to the ethyl alcoholic solution, in glistening leaflets. (Found: Cl′, 11·2. $C_{19}H_{22}N_2$, HCl requires Cl′, 11·3 %.) $[\alpha]_D^{19} - 3{\cdot}95°$ ($c = 1{\cdot}012$ in water).

Desoxydihydrocinchonine. Cinchonine chloride (4 g.) in *N*-sulphuric acid (40 c.c.) was treated with a catalyst (5 g.) consisting of approximately ten parts of "suchar" charcoal and one part of palladium chloride and the mixture shaken with access of hydrogen at a pressure of about 100 cm. of water. After about 650 c.c. of hydrogen had been absorbed in 30 min. absorption became very slow. This volume indicates reduction of the vinyl group and replacement of the chlorine by hydrogen. The precipitate from the basified solution crystallized from aqueous acetone as the dihydrate, in plates, m.p. 62°. $[\alpha]_D^{27} + 141{\cdot}8°$ ($c = 1{\cdot}00$ in ethyl alcohol). Jacobs and Heidelberger (1922) give m.p. 60° and $[\alpha]_D^{30} + 113{\cdot}8°$ for the anhydrous base, whilst Rabe (1932) gives $[\alpha]_D^{20} + 147°$ for the anhydrous base in absolute ethyl alcohol.

The hydrochloride crystallized from methyl alcohol and ether in prisms, m.p. 192°, to a yellow liquid. (Found: Cl′, 11·2; Calc. Cl′, 11·2 %.) $[\alpha]_D^{26} + 71{\cdot}4°$ ($c = 1{\cdot}0$ in water). Jacobs and Heidelberger (1922) give m.p. 197–199° and $[\alpha]_D^{24} = 69{\cdot}3°$ ($c = 1{\cdot}07$ in water).

Desoxydihydroquinidine was prepared from hydroquinidine chloride by catalytic reduction, the absorption of hydrogen only corresponding to removal of the halogen and replacement by hydrogen. The base crystallized from aqueous acetone in rhombs, m.p. 81–83°, in agreement with the data of Jacobs and Heidelberger (1920).

Partial catalytic reduction of quinidine chloride. Quinidine chloride hydrochloride (5 g.) in water (60 c.c.) was hydrogenated with 1 g. of catalyst. When about one-half of the hydrogen required for replacement of chlorine and reduction of the vinyl group had been absorbed, the process was interrupted and one-half the reaction fluid was removed and the ionized chlorine determined in an aliquot part. Calculation from the ionized chlorine and the hydrogen consumption showed that replacement of organically bound chlorine and reduction of the vinyl group were proceeding simultaneously. The remainder of the original solution was completely hydrogenated, consuming in the process the calculated amount of hydrogen and when basified gave desoxydihydroquinidine.

Desoxydihydrocinchonidine. Cinchonidine chloride (3·1 g.) was dissolved in N-sulphuric acid (40 c.c.) and hydrogenated in the presence of 2 g. of the palladized charcoal catalyst. In 30 min. approximately 500 c.c. of hydrogen were absorbed, corresponding to two molecular proportions, after which absorption ceased. The base was not obtained crystalline but was converted into the *monohydrochloride* which crystallized from methyl alcohol and ether in clusters of flattened needles, m.p. 186–188°. (Found on vacuum dried material: Cl′, 11·1; N, 8·6. $C_{19}H_{24}N_2$, HCl requires Cl′, 11·2; N, 8·9 %.) $[\alpha]^{21}_{D} + 9{\cdot}0°$; $[\alpha]^{21}_{5461} + 11{\cdot}1°$ ($c = 1{\cdot}08$ in water).

Quinene was prepared by the method of Comstock and Königs (1884), purification being effected through the cuprichloride (Cohen 1933). When the base was neutralized to litmus in hot aqueous solution with hydrochloric acid a monohydrochloride was not obtained on cooling but instead the base separated, the mother liquor becoming acid in reaction. The stable dihydrochloride obtained by adding the requisite amount of hydrochloric acid was crystallized from methyl alcohol and ether and separated in fine yellow needles, m.p. 180–185°. (Found: Cl′, 18·3; Calc. Cl′, 18·7 %.) Giemsa and Halberkann (1921) give m.p. 182° and describe this as a neutral salt although its solution is acid to litmus.

Hydroquinene was prepared from hydroquinine chloride and isolated as the cuprichloride (Cohen 1933). It gave the base from aqueous acetone in stout prisms, m.p. 78–80°, in agreement with Giemsa and Halberkann's data. The dihydrochloride separated from methyl alcohol on addition of ether as a dihydrate, fine yellow needles, m.p. 215–218°, to a red liquid. (Found: Loss at 110°, 8·8. $C_{20}H_{24}ON_2$, 2HCl, $2H_2O$ requires H_2O, 8·6 %. On anhydrous material Cl′, 18·3; Calc. Cl′, 18·6 %.) $[\alpha]^{22\cdot5}_{D} + 33{\cdot}9°$; $[\alpha]^{22\cdot5}_{5461} + 40{\cdot}5°$ ($c = 0{\cdot}974$ in water).

Cinchene from cinchonine chloride was isolated as the cuprichloride which gave a base, m.p. 123°, from aqueous acetone in agreement with Comstock and Königs (1884). Like quinene, cinchene does not give a monohydrochloride but a mixture of base and dihydrochloride. The *dihydrochloride* crystallized from methyl alcohol and ether in fine woolly needles, m.p. 185–190°, which are very hygroscopic. (Found: Cl′, 18·5. $C_{19}H_{20}N_2$, 2HCl, $2H_2O$ requires Cl′, 18·4 %.)

Methylquitenine was prepared in the usual way and isolated from the esterification fluid as the *dihydrochloride*. It was purified by crystallization from 3 vol. of boiling *iso*-propyl alcohol and separated in silky needles, which have no definite m.p. but effervesce between 110° and 120°. (Found (micro): C, 49·5; H, 6·7; N, 5·5, 5·6; Cl, 14·1, 14·4; H_2O, 11·1. $C_{20}H_{24}O_4N_2$, 2HCl, $3H_2O$ requires C, 49·7; H, 6·7; N, 5·8; Cl, 14·7; H_2O, 11·1 %.) The *dihydrobromide trihydrate* crystallizes from dilute hydrobromic acid in prismatic needles. (Found: Loss at 100°, 9·7. $C_{20}H_{24}O_4N_2$, 2HBr, $3H_2O$ requires H_2O, 9·4 %. Found in dried solid: Br, 30·5. $C_{20}H_{24}O_4N_2$, 2HBr requires Br, 30·8 %.) The anhydrous material softens above 180°, effervescing at 205°, and has $[\alpha]_D^{20} - 200{\cdot}6°$; $[\alpha]_{5461}^{20} - 249°$ ($c = 1{\cdot}003$ in water).

Quitenamide. Two lots of 5 g. each of methylquitenine dihydrochloride were added to chilled concentrated ammonia (50 c.c. d.0·88) in pressure bottles and then heated at 60° for 15 hr. The amorphous base gradually dissolved and towards the end a crystalline substance separated in stout needles. This was collected and washed with dilute sodium hydroxide to remove quitenine, yield 3·2 g. It was recrystallized from 160 parts of boiling water, from which it crystallized in prisms and sometimes in hexagonal plates, m.p. 203°. (Found (micro): Loss at 120°, 1·7; C, 64·9; H, 6·9; N, 11·8, 11·9, 12·3. $C_{19}H_{23}O_3N_3$, $\frac{1}{2}H_2O$ requires H_2O, 2·6; C, 65·1; H, 6·9; N, 12·0 %.) $[\alpha]_D^{20} - 333{\cdot}6°$; $[\alpha]_{5461}^{20} - 410{\cdot}6°$ ($c = 0{\cdot}96$ (anhydrous base) in 0·1 *N*-hydrochloric acid). The ammoniacal mother liquors contain much quitenine. *Quitenamide hydrochloride dihydrate* separates from the neutral aqueous solution in sheaves of needles, melting indefinitely between 155° and 200°, but when dried at 110° it sinters at 197° and melts at 200°. (Found: Loss at 110°, 8·8. $C_{19}H_{23}O_3N_3$, HCl, $2H_2O$ requires H_2O, 8·7 %. Found in dried salt: Cl′, 9·0; N (micro), 10·8. $C_{19}H_{23}O_3N_2$, HCl requires Cl′, 9·3; N, 11·1 %.) $[\alpha]_D^{15} - 206{\cdot}8°$; $[\alpha]_{5461}^{15} - 250{\cdot}1°$ ($c = 1{\cdot}00$ in water).

Quitenmethylamide. Amorphous quitenine methyl ester (5 g.) was heated with aqueous methylamine (50 c.c. 30 % w/v) in a pressure bottle at 50° for several hours. The clear solution was evaporated to small bulk and the solid, which separated at low temperature, collected and washed with dilute sodium hydroxide, yield 2·1 g. On crystallizing from 10 % alcohol, *quitenmethylamide tetrahydrate* separates in glistening leaflets, m.p. 90°, effervescing at 100°, resolidifying and melting finally at 250°. (Found: Loss at 110° or at 80° over P_2O_5 *in vacuo*, 16·8; C (micro), 55·9; H, 7·8; N, 9·7. $C_{20}H_{25}O_3N_3$, $4H_2O$ requires H_2O, 16·9; C, 56·2; H, 7·8; N, 9·8 %.) $[\alpha]_D^{20} - 179{\cdot}3°$; $[\alpha]_{5461}^{20} - 219{\cdot}2°$ ($c = 1{\cdot}01$ in ethyl alcohol). Quitenmethylamide is readily soluble in methyl or ethyl alcohol but requires about 115 parts of boiling water for solution.

Methylcinchotenine dihydrochloride monohydrate was obtained by esterification of cinchotenine in methyl alcohol saturated with hydrogen chloride. It was recrystallized by careful addition of acetone to its solution in 4 vol. of methyl alcohol and separated in large diamond or hexagonal-shaped plates, m.p. about 253° (with decomp.). (Found (micro): H_2O, 4·7; N, 6·8; Cl, 17·4. $C_{19}H_{22}O_3N_2$, 2HCl, H_2O requires H_2O, 4·3; N, 6·7; Cl, 17·0%.) The free base crystallizes from 44 vol. of boiling methyl alcohol in prisms, m.p. 250–252°. Jacobs and Heidelberger (1922) give m.p. 243–244·5°. (Found (micro): C, 69·9; H, 6·7; N, 8·6; Calc. C, 69·9; H, 6·8; N, 8·6%.)

Methylcinchotenidine dihydrochloride was prepared similarly. It is very soluble in the usual organic solvents and can only be recrystallized with difficulty. From butyl alcohol, with or without the addition of acetone, it separates in circular compact clusters, m.p. 235° (decomp.), which are anhydrous. (Found (micro): Cl, 18·0, 17·8, 17·8; N, 7·2. $C_{19}H_{22}O_3N_2$, 2HCl requires Cl, 17·8; N, 7·0%.) The base *methylcinchotenidine* crystallizes from 6 vol. of benzene in silky needles, which when freshly prepared melt at 95–98°, resolidify at a higher temperature and then melt towards 150°, but when dried in a vacuum melt at 152–153°. (Found (micro): On freshly prepared solid, loss in a vacuum over H_2SO_4, 16·2; loss at 97°, 31·5. On vacuum dried solid, N, 7·0. On solid dried at 97°, C, 69·3; H, 6·5. $C_{19}H_{22}O_3N_2$, $2C_6H_6$ losing $1C_6H_6$ requires loss, 16·4; losing $2C_6H_6$ requires loss, 32·4; $C_{19}H_{22}O_3N_2$, C_6H_6 requires N, 6·9; $C_{19}H_{22}O_3N_2$ requires C, 69·9; H, 6·8%.)

γ-Phenylimido-α-keto-α-(3-pyridyl) butane (II). β-Pyridoylacetone (24·3 g.) was heated with aniline (15 g.) for 3 hr. at 100°. The solid product, 35·6 g., melted at 76–79° and on crystallization from 20 vol. of ligroin separated in small plates, m.p. 82–83°. (Found (micro): C, 75·6; H, 5·9. $C_{15}H_{14}ON_2$ requires C, 75·6; H, 5·9%.)

2-*Methyl*-4-(3-*pyridyl*) *quinoline* (III). The above anil (18·6 g.) was finely powdered and added gradually to sulphuric acid (93 g.) with mechanical stirring. The clear solution was heated at 100° for 3 hr. and when cold poured on to ice. The alkalized solution was extracted with ether, which on evaporation left a mixture of bases (12·2 g.). This was separated by fractional distillation under reduced pressure into aniline and 2-*methyl*-4-(3-*pyridyl*) *quinoline* (6·9 g.) boiling between 224° and 250° (bath temperature) at 15 mm. pressure. It readily solidified and crystallized from 8 vol. of boiling ligroin in square plates, m.p. 108–109°. (Found (micro): C, 81·7; H, 5·3; N, 12·8. $C_{15}H_{12}N_2$ requires C, 81·8; H, 5·5; N, 12·7%.) The *dihydrochloride*, m.p. 288° (eff.), prepared by evaporating the base to dryness with excess of hydrochloric acid is extremely readily soluble in water with an acid reaction to Congo-paper. It is best recrystallized from methyl alcohol with addition of dry ether. (Found (micro): C, 54·0; H, 5·5; N, 8·2. $C_{15}H_{12}N_2$, 2HCl, $2\frac{1}{4}H_2O$ requires C, 53·6; H, 5·5; N, 8·4 %.) The *monopicrate* crystallizes from 300 parts of boiling water in needles, m.p. 197–198°. (Found (micro): C, 56·4; H, 3·4; N, 15·5. $C_{15}H_{12}N_2$, $C_6H_3O_7N_3$ requires C, 56·1; H, 3·4; N, 15·6 %.) The *dipicrate* readily loses a part of its picric acid on crystallization and is therefore best prepared in presence of 50 % excess of picric acid. It then crystallizes homogeneously in long needles, m.p. 205°, and is slightly more soluble in boiling water than the monopicrate. (Found (micro): C, 47·8; H, 2·7; N, 16·6. $C_{15}H_{12}N_2$, $2C_6H_3O_7N_3$ requires C, 47·8; H, 2·7; N, 16·5 %.)

2-*Styryl*-4-(3-*pyridyl*) *quinoline* (IV). The pyridyl-quinaldine described above (1·0 g.) was boiled with benzaldehyde (5 c.c.) for 2 hr., the product acidified and benzaldehyde removed by ether extraction. The acid solution, made alkaline and extracted with ether, gave the styrylpyridyl-quinoline (1·3 g.) as a readily crystallizing base. From organic solvents it crystallizes readily, from benzene in large plates, m.p. 167–168°. (Found (micro): C, 85·6; H, 5·3; N, 9·1. $C_{22}H_{16}N_2$ requires C, 85·7; H, 5·2; N, 9·1 %.)

SUMMARY

Experimental bird malaria is utilized for following the effect of changes of structure in the cinchona alkaloids on the therapeutic activity. It is shown that although antiplasmodial action is not very sensitive to stereochemical changes, it is sensitive to changes of structure. Thus there is apparent loss of activity when the central —CHOH— group is modified in any way. Thus its conversion into —CHCl—, $—CH_2—$ or —CH= leads in almost all cases to an increase in toxicity and loss of antiplasmodial action. The preparation and properties of a series of allied substances is also described.

We desire to express our thanks to Miss N. Vincent and Dr Parr Tate, working in the laboratory of Professor D. Keilin, F.R.S., at the Molteno Institute, Cambridge, for the biological results recorded in this paper.

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Antiplasmodial action and chemical constitution

Part II. Some simple synthetic analogues of quinine and cinchonine

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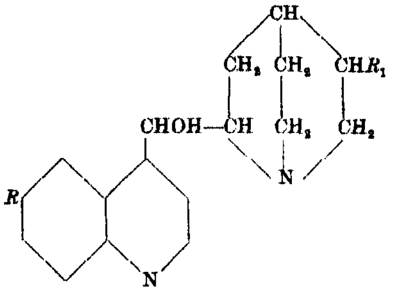
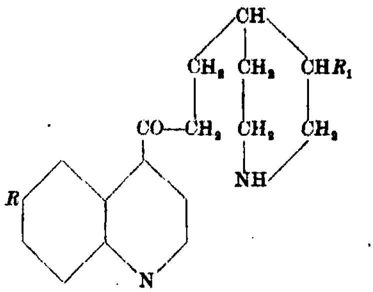
In Part I (Cohen and King 1938) it was shown that retention of the bridge carbinol group in the molecule of the cinchona alkaloids (I) was essential for antiplasmodial activity, as tested on bird malaria. Now Giemsa and Oesterlin (1933) have shown that quinicine (II, $R = \text{OMe}$; $R_1 = .\text{CH:CH}_2$), an isomeride of quinine, is devoid of antiplasmodial action, and it seemed possible that reduction to the carbinol (III), which occurs in two diastereoisomeric forms, might restore activity, since the pictorial resemblance between the dihydroquinicinols (III, $R = \text{OMe}$; $R_1 = .\text{CH}_2.\text{CH}_3$) and quinine (I, $R = \text{OMe}$; $R_1 = .\text{CH:CH}_2$) could hardly be greater. Both carbinols which were made according to the method of Heidelberger and Jacobs (1922) proved to be inactive (Table I), as did a methylation product, *d*-*N*-methyldihydroquinicinol (IV).

From this result it seemed essential that the piperidine ring system, which is still present in (III), should be as near the quinoline ring system



(I)



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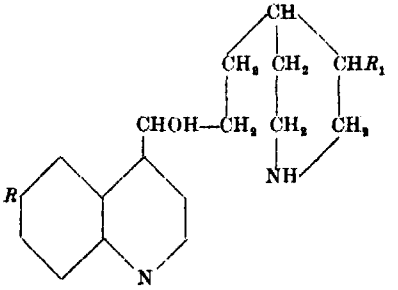

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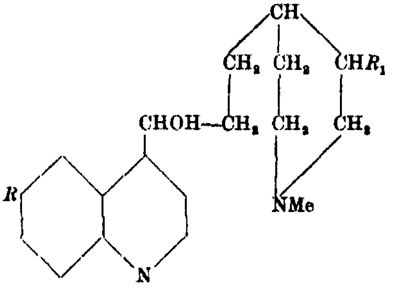
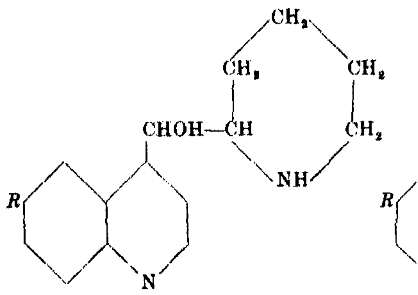


(IV)

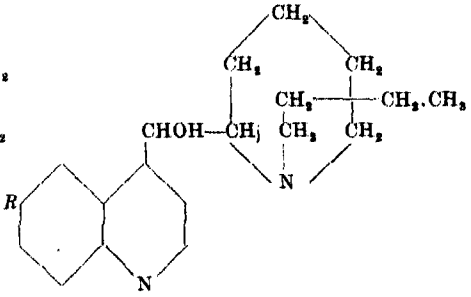
TABLE I

Substance	Dose in mg. per 20 g. of body weight	Day of appearance of parasites in blood	Remarks
l-Dihydroquinicinol 2HCl	6 × 1·25	8	—
	6 × 1·25	6	M.T.D.
d-Dihydroquinicinol 2HCl	6 × 1·25	8	M.T.D.
	6 × 0·3	6	—
	6 × 1·875	6	—
d-*N*-Methyldihydroquinicinol 2HCl	6 × 0·625	5	M.T.D.
Control birds	—	5–6	—

as possible, consistent with retention of the carbinol group. If, therefore, any further progress was to be made in unravelling the factors necessary for antiplasmodial action, it was eminently desirable that substances of the type of (V) should be synthesized, where *R* is H or MeO. An *N*-butyl derivative of such a substance (VI) would be structurally very similar to quinine (I, $R = \text{OMe}$; $R_1 = .\text{CH}:\text{CH}_2$) and would only differ in molecular weight from it by four hydrogen atoms. The synthesis of such ring systems should, at first sight, not be difficult by following the methods worked out by Rabe for the synthesis of the hydro-cinchona alkaloids. However, Ruzicka (1921) and his co-workers (1924) had already attempted such

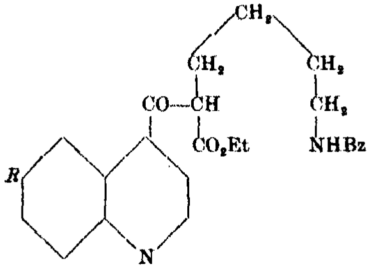


(V)

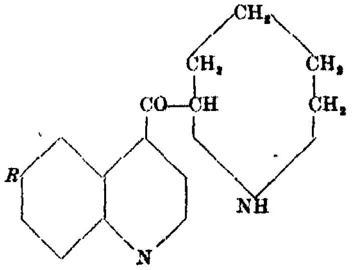


(VI)

syntheses but with little ultimate success. A careful perusal of their papers shows that three factors had contributed to their lack of success. In the first place the yields of primary condensation product of the type of (VII) and at other stages were very small. Secondly, the intermediate ketones of the type (VIII) were sensitive substances, very difficult to



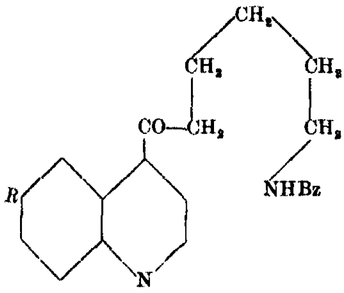
(VII)



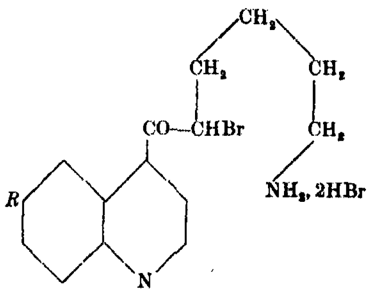
(VIII)

obtain in a state of purity, and thirdly, when obtained they were difficult to reduce to the carbinol (V). However, inspired by the urgent necessity of obtaining substances of the type of (V) and (VI), if any advance was to be made, we reopened the subject and after overcoming unforeseen obstacles at almost every stage of our work, we have finally succeeded in synthesizing *4-quinolyl-α-piperidylcarbinol* (V, $R = \mathrm{H}$), its methoxy derivative, 4-(6-*methoxyquinolyl*)-*α-piperidylcarbinol* (V, $R = \mathrm{OMe}$), and a number of their *N*-alkyl derivatives. For this purpose it was essential to have the starting materials, ethyl ε-benzamidocaproate, ethyl cinchoninate and ethyl quininate, available in quantity and improved alternative processes have been worked out for attaining this object.

The condensation of ethyl ε-benzamidocaproate, $\mathrm{EtOOC.(CH_2)_5.NHBz}$, and ethyl cinchoninate, using sodium ethoxide, was found by Ruzicka, Seidel and Liebl (1924) to give a 17 % yield of condensation product (VII, $R = \mathrm{H}$). By replacing the sodium ethoxide by sodium and still more so by sodamide free from nitrite, we have found that the yield of condensation product from ethyl cinchoninate can be raised to 64 % and from ethyl quininate to 36 %. The condensation products (VII, $R = \mathrm{H}$ or OMe) were oils and were not further characterized but were hydrolysed by careful digestion with 17 % hydrochloric acid to stable crystalline substances, namely, *ε-benzamidoamyl-4-quinolyl ketone* and *ε-benzamidoamyl-4-(6-methoxyquinolyl) ketone* respectively (IX, $R = \mathrm{H}$ or OMe). These



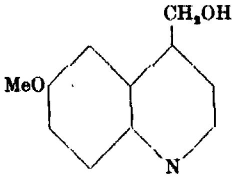
(IX)



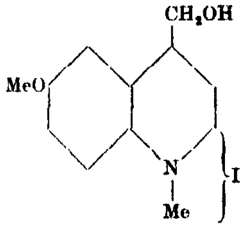
(X)

ketones, by more drastic hydrolysis, were converted into oily aminoketones which were straightway mono-brominated in hydrobromic acid solution to yield crystalline, stable α-bromo-ε-aminoamyl-4-quinolyl ketone dihydrobromide (X, $R = \mathrm{H}$), and the corresponding 6-*methoxy* derivative (X, $R = \mathrm{OMe}$) in good yield. For the removal of organically bound bromine in substances of these types, Ruzicka, Seidel and Liebl (1924) used sodium ethoxide in alcoholic solution, but with unsatisfactory results. We tried out the process and found it unworkable owing to the lability of the products of the reaction. The difficulties were eventually overcome by shaking the bromo-dihydrobromide (X) in saturated sodium carbonate solution in the presence of ether until the whole of the bromine had become ionized, usually for 20 min. The ethereal solution of the cyclized base and other products was rapidly evaporated at room temperature by means of a pump, the residual syrups straightway dissolved in methyl alcohol and reduced catalytically with hydrogen and platinum oxide to the carbinol. Ruzicka, Seidel and Liebl (1924), fearing simultaneous reduction of the quinoline nucleus, used sodium ethylate and aluminium for the reduction, and to this, in particular, must be ascribed their general lack of success in the final stage of the preparation of the carbinols.

4-*Quinolyl-α-piperidylcarbinol* (V, $R = \mathrm{H}$) and 4-(6-*methoxyquinolyl*)-*α-piperidylcarbinol* (V, $R = \mathrm{OMe}$) are stable crystalline solids which form crystalline neutral *monohydrochlorides*, but acid *dihydrochlorides*. During the reduction of the intermediate labile ketones a new asymmetric centre appears and both these carbinols should occur in two diastereoisomeric forms. In the case of the preparation of 4-quinolyl-α-piperidylcarbinol (V, $R = \mathrm{H}$) there is a considerable amount of by-product formed, but no isomeric base has been obtained from it in a crystalline state; in the case, however, of the corresponding methoxy derivative, an isomeride, iso-4-(6-*methoxyquinolyl*)-*α-piperidylcarbinol*, was isolated on one occasion. Another crystalline by-product of the catalytic reduction of 4-(6-methoxyquinolyl)-α-piperidyl ketone (VIII, $R = \mathrm{OMe}$) was 6-*methoxyquinolyl-4-carbinol* (XI),



(XI)

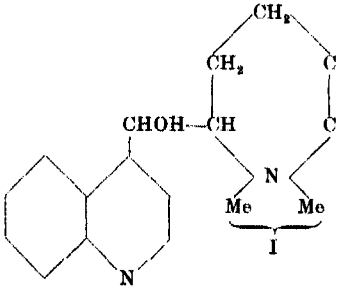


(XII)

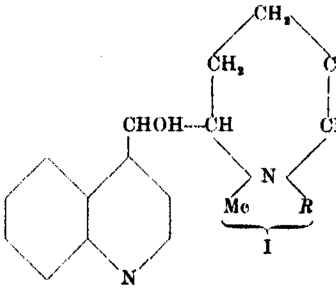
which crystallizes as a dihydrate, but forms an anhydrous *methiodide* (XII). The second scission product of the molecule should be piperidine, and although it was not isolated, its odour was frequently observed when the mother liquors were basified and the bases extracted.

The *N*-alkylation of 4-quinolyl-α-piperidylcarbinol and 4-(6-methoxyquinolyl)-α-piperidylcarbinol (V) presented many difficulties. This is due to the fact that quaternary salt formation from the primarily formed *N*-alkyl derivative was very rapid. There was, however, very little tendency to quaternary salt formation on the quinoline nucleus, an observation in keeping with the behaviour of the cinchona alkaloids. The higher alkyl iodides from ethyl onwards are not very reactive substances, and the raised temperatures necessary to make them react with the secondary nitrogen atom of the piperidylcarbinols so greatly accelerated quaternary salt formation that it has hitherto proved impossible to isolate the *N*-alkyl derivatives from *N*-ethyl onwards by direct alkylation.

Since quaternary salt formation was such a predominant feature of these alkylations, it seemed possible that Clarke's (1913) process of degradation of quaternary salts to the tertiary bases by the action of heat on the quaternary methohydrosulphides might be adapted not only to the preparation of the *N*-methyl derivatives, but also of higher *N*-alkyl derivatives. With this end in view, *4-quinolyl-α-N-methylpiperidylcarbinol methiodide* (XIII) was prepared and converted into the methohydrosulphide which was heated in the vacuum of a water pump at 100° until evolution of dimethylsulphide had ceased. The residual base crystallized readily in hexagonal tablets, and proved to be *4-quinolyl-α-*N*-methylpiperidylcarbinol*, m.p. 120–121°. Ruzicka, Seidel and Liebl (1924) have described a carbinol to which this constitution is assigned as a fine crystalline powder, m.p. 113–114°. It seems probable that on our nomenclature the authors had in hand the diastereoisomeric *iso-* base, especially as the mode of reduction employed by these authors, aluminium, alcohol and sodium hydroxide, might have favoured the production of the *iso-* series.



(XIII)

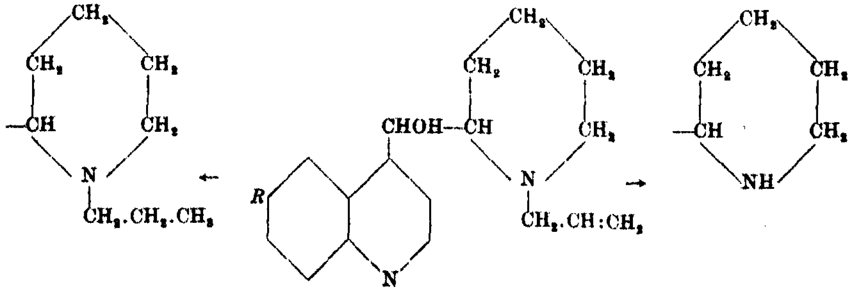


(XIV)

The possibility of adapting Clarke's process for the production of higher *N*-alkyl derivatives has been only partly explored. 4-Quinolyl-α-*N*-methylpiperidylcarbinol fairly readily adds on ethyl, allyl or crotyl iodide with the formation of the corresponding quaternary salts (XIV, $R = .CH_2.CH_3$; $.CH_2.CH{:}CH_2$; $.CH_2.CH{:}CH.CH_3$, respectively), but we have not yet had the opportunity of determining whether these mixed quaternary salts, as their hydrosulphides, would preferentially part with dimethylsulphide leaving the higher *N*-alkyl bases. The success of the method as a preparative method will naturally depend on the availability of the tertiary *N*-methyl base.

In the meantime a method of circumventing the slow reactivity of the higher saturated alkyl iodides was devised by making use of the much greater reactivity of the corresponding unsaturated derivatives such as allyl iodide and crotyl iodide, for it is known that allyl iodide and methyl iodide are comparable in their reactivity with tertiary amines, whereas ethyl and propyl iodides are very much less reactive (Preston and Jones 1912). In agreement with this it has been found that 4-quinolyl-α-piperidylcarbinol and 4-(6-methoxyquinolyl)-α-piperidylcarbinol will react with methyl, allyl or crotyl iodides at room temperature in acetone solution, with the production of the corresponding mono-*N*-alkyl derivative. The reaction is best carried out either in the presence of solid sodium bicarbonate or in the presence of one molecular proportion in excess of the secondary base to be alkylated; in either case mixtures are formed. Both in the quinolyl and in the methoxyquinolyl series, for the successful isolation of the products of the reaction, use has been made of the property that the hydriodides of the starting bases and the bases themselves are respectively less soluble than the hydriodides of the tertiary bases and the tertiary bases themselves. In this way the N-*methyl*, N-*allyl* and N-*crotyl* derivatives of 4-*quinolyl-α-piperidylcarbinol* and 4-(6-*methoxyquinolyl*)-*α-piperidylcarbinol* have been obtained both in the form of crystalline bases and as their salts in sufficient quantity for analysis and for tests on their antiplasmodial action.

The allyl and the crotyl bases have also served for the preparation of the saturated *N*-propyl and *N*-butyl derivatives in both series. It was unexpectedly found that when the *N*-allyl or *N*-crotyl bases were reduced in methyl alcohol with hydrogen and platinum oxide a considerable proportion of the *N*-allyl and *N*-crotyl groups was lost with regeneration of the parent secondary bases, but it has been found possible to isolate sufficient of the very soluble N-*propyl* and N-*butyl* derivatives of 4-*quinolyl-α-piperidylcarbinol* and 4-(6-*methoxyquinolyl*)-*α-piperidylcarbinol* either as bases or salts for their characterization and for antiplasmodial tests. The catalytic hydrogenation evidently takes the two courses shown:



It is probable that the *N*-ethyl derivatives could be prepared through the *N*-vinyl derivatives by making use of the reactivity of vinyl iodide or bromide, but this has not yet been tried experimentally.

The results of the tests, on bird malaria due to *Plasmodium relictum* (= *Pl. praecox*) in canaries, of 4-quinolyl-α-piperidylcarbinol, 4-(6-methoxyquinolyl)-α-piperidylcarbinol, and their *N*-alkyl derivatives as hydrochlorides, are shown in the following table:

Substance	Dose in mg. per 20 g. of body weight	Day of appearance of parasites in blood	Remarks
4-Quinolyl-α-piperidylcarbinol	6 × 10	5	—
	6 × 20	5	—
N-Methyl derivative	1 × 10 + 5 × 5	5	—
	1 × 10 + 5 × 2·5	6	—
N-Allyl derivative	1 × 10 + 5 × 5	5	M.T.D.
	1 × 10 + 5 × 5	6	—
N-Crotyl derivative	1 × 5 + 5 × 2·5	5	M.T.D.
	1 × 5 + 5 × 2·5	5	—
N-Propyl derivative	2 × 5 + 4 × 2·5	5	M.T.D.
	2 × 5 + 4 × 2·5	5	—
N-Butyl derivative	4 × 2·5 + 2 × 1·25	5	M.T.D.
	6 × 2·5	5	M.T.D.
4-(6-Methoxyquinolyl)-α-piperidylcarbinol	6 × 5	Sterile	—
	6 × 5	,,	—
	6 × 2·5	10	—
	6 × 2·5	7	—
	6 × 1·25	7	—
	6 × 1·25	6	—
N-Methyl derivative	5 × 5 + 1 × 2·5	7	M.T.D.
N-Allyl derivative	5 × 5 + 1 × 2·5	5	M.T.D.
N-Crotyl derivative	6 × 2·5	7	—
N-Propyl derivative	3 × 5 + 3 × 2·5	5	M.T.D.
	1 × 5 + 5 × 2·5	5	—
N-Butyl derivative	1 × 2·5 + 5 × 1·25	6	M.T.D.
	1 × 2·5 + 5 × 1·25	5	M.T.D.
iso-4-(6-Methoxyquinolyl)-α-piperidylcarbinol	6 × 10	10	—
	6 × 10	12	M.T.D.
Hydrocinchonine	6 × 5	5	M.T.D.
Quinine	6 × 2·5	12	—
Control birds	—	5–7	—

A perusal of this table shows that no quinolyl derivative without the 6-methoxy group has any activity. Even 4-quinolyl-α-*N*-crotylpiperidylcarbinol, which is isomeric with hydrocinchonine or hydrocinchonidine, shows no activity. On the strain of *Pl. praecox* used, hydrocinchonine itself had no activity, although Buttle, Henry and Trevan (1934) found it to be active on their strain, but at the same time to be one of the weakest of the cinchona alkaloids.

On the other hand, the two parent isomeric 6-methoxy bases both showed activity and 4-(6-methoxyquinolyl)-α-piperidylcarbinol, which is the parent of the series of alkyl derivatives shown, proved to be the more active isomeride. Professor D. Keilin, F.R.S., informs us that 4-(6-methoxyquinolyl)-α-piperidylcarbinol has about the same M.T.D. as quinine but is about one-half as effective in terms of the therapeutic index. It is surprising that all the *N*-alkyl derivatives in the methoxy series show no activity, although addition of the alkyl groups makes the two nitrogen atoms tertiary as in quinine and increases the molecular weight, the *N*-crotyl derivative being isomeric with dihydroquinine. For this loss of activity we cannot advance any real explanation. It is of passing interest that the toxicity of this whole group of substances is comparable with the toxicity of the cinchona alkaloids and not with substances of the type of plasmochin, which are very much more toxic. Finally, it may be pointed out that the two methoxy bases with antiplasmodial activity appear to be the first compounds to be synthesized on the pattern of the quinine molecule which show any activity in bird malaria.

EXPERIMENTAL

α-Chloro-ε-benzamidopentane

For the successful preparation of α-chloro-ϵ-benzamidopentane by the action of phosphorus pentachloride on benzpiperidide by von Braun's process (1904) it is essential to use a pure benzpiperidide free from partially reduced pyridine derivatives, which are invariably present in commercial piperidine.

N-*Benzpiperidide*. Piperidine (100 g.) was dissolved in water (460 c.c.), sodium hydroxide pellets (64·5 g.; 1·3 mol.) added, the solution cooled below 40° and benzoyl chloride (168 g.) added slowly with vigorous stirring during 1·5 hr. After the addition of the benzoyl chloride was complete, the temperature was raised to 40° for 30 min. The heavy oil which had separated was extracted with benzene and the extract washed with water and finally distilled. Rectification of the residual oil gave 196 g. of crude benzpiperidide, b.p. 184° at 17 mm.

N-Benzpiperidide (196 g.) was dissolved in acetone (1500 c.c.) and powdered potassium permanganate (80 g.) added in small amounts over a period of 10–12 hr. During this process the solution was surrounded by cold water and was vigorously stirred. The end point of the oxidation was taken when there was a permanency of pink colour for 30 min. after addition of about 0·5 g. of permanganate. The manganese oxides were removed, the acetone distilled off and the residue fractionated. The yield of pure benzpiperidide was 139 g., b.p. 184–186° at 17 mm.

α-Chloro-ε-benzamidopentane. *N*-Benzpiperidide (100 g.) and phosphorus pentachloride (112·6 g.; 1 mol.) were heated gently under reflux until reaction commenced. The heating was discontinued until the vigorous ebullition had ceased and then the mixture was refluxed gently for 30 min. After cooling, the dark brown liquid was decomposed by ice, 50 % sodium hydroxide solution added until the liquor was only just acid to Congo-paper and finally the mixture was steam distilled until no more volatile oil passed over. The residue liquors in the flask were then rapidly chilled and shaken vigorously, when α-chloro-ε-benzamidopentane was obtained as a pale brown granular mass, yield 90 g.

Using pure benzpiperidide the α-chloro-ε-benzamidopentane obtained as above was sufficiently pure for direct conversion to α-cyano-ε-benzamidopentane. If, however, the benzpiperidide contained any partially reduced pyridines, the α-chloro-ε-benzamidopentane obtained after reaction with phosphorus pentachloride could not be induced to solidify and extraction with ether was necessary, followed by crystallization of the product from a mixture of ether and ligroin. In the latter circumstances, the yields were very erratic and did not exceed 50 %.

α-Cyano-ε-benzamidopentane. α-Chloro-ε-benzamidopentane (90 g. crude product as obtained in the previous preparation) was dissolved in alcohol (500 c.c.), a solution of potassium cyanide (110 g.) in water (220 c.c.) added, and the whole boiled under reflux for 14 hr. (cf. Ruzicka 1921). The greater part of the alcohol was then distilled off, water (500 c.c.) was added to the mixture, which was rapidly cooled and shaken, when the crude dark brown α-cyano-ε-benzamidopentane separated as a crystalline powder, yield 74 g.

Ethyl ε-benzamidocaproate. The above described cyano-compound (74 g.) was suspended in absolute alcohol (400 c.c.) and the solution saturated with hydrogen chloride at room temperature (cf. Ruzicka 1921). Towards the end of the process ammonium chloride began to separate and the amount increased considerably during the subsequent 4 hr. boiling. The greater part of the alcohol was then distilled, the residue diluted with water, cooled and extracted with ether. The ethereal solution was washed with dilute sodium carbonate solution and finally with water. Removal of the ether and distillation of the residue *in vacuo* gave ethyl ϵ-benzamidocaproate as an almost colourless oil, b.p. 204° at 1 mm., which readily solidified on cooling, yield 54 g.

The total yield of this ester from 1000 g. of commercial piperidine, put through the process in 100 g. batches, was 741 g.

Ethyl cinchoninate

For the preparation of this ester, three processes have been thoroughly worked out, starting from quinoline, aniline or isatin. All three processes are described, because in their application to other quinolines the process chosen will depend on the availability of the respective starting materials.

(*a*) *From quinoline*

The original process was described in a series of papers by Kaufmann and his co-workers. The following is essentially Kaufmann's process worked out to give the maximum yields at each stage.

Synthetic quinoline was converted quantitatively into methylquinoline methosulphate by heating together equimolecular proportions of the components in benzene for 30 min. The crystalline product was used for the next process in most cases, but when coloured was converted into the iodide by double decomposition with two molecular proportions of potassium iodide in a small volume of water.

N-*Methyl*-4-*cyanoquinolane*. Methylquinoline methosulphate (22·4 g.), or an equivalent of the iodide, was dissolved in water (40 c.c.), ether (1 c.c.) added, and then a solution of potassium cyanide (7·6 g. of 98–99 %) in water (15 c.c.). The whole was mechanically shaken for 2 hr. and the yellow crystalline solid collected and washed with a little ether, yield 14·0 g. (94 %), m.p. 78°. Kaufmann and Albertini (1909) give 80°. This quinolane is unstable, especially so when dried over sulphuric acid in a vacuum. It was usually dried for a few hours in a vacuum over sodium hydroxide and then straightway used for the next process.

4-*Cyanoquinoline methiodide*. The above quinolane (14 g.) was dissolved in dry pyridine (28 c.c.) and poured into a solution of iodine (18·4 g.) in absolute alcohol (148 c.c.). After cooling at 0° overnight, the dark red crystalline solid was collected, yield 19·2 g., m.p. 209°. It was crystallized from water by treating with sufficient water at 50° to dissolve the crystalline material and leave a red-brown amorphous solid undissolved. On filtration and cooling, pure 4-cyanoquinoline methiodide was obtained, m.p. 227–228°, whereas Kaufmann and Widmer (1911) give m.p. 216°. In a similar manner from 380 g. of crude iodide there were obtained 289 g. of pure iodide.

4-*Cyanoquinoline.* Pure 4-cyanoquinoline methiodide (25 g.) was heated in a distilling flask for 30 min. at 220° in a metal bath. Methyl iodide distilled off and was collected. The residue was dissolved in 3*N*-hydrochloric acid (250 c.c.), filtered and made just alkaline. The precipitated crystalline cyanoquinoline was collected, yield 82–85 %. Batches of 50 g. of iodide could be put through the same process without affecting the yield.

Cinchoninic acid and ethyl ester. 4-Cyanoquinoline was hydrolysed by boiling with 2 vol. of 70 % sulphuric acid for 2 hr. The dark brown solution was cooled, diluted with water, made neutral to Congo-paper with ammonia and the precipitated cinchoninic acid collected. From 414·5 g. of cyanoquinoline there were obtained 406·3 g. (87 % yield) of cinchoninic acid. The acid (10 g.) was refluxed with a mixture of absolute alcohol (50 c.c.) and concentrated sulphuric acid (8 c.c.) for 5 hr. The greater part of the alcohol was distilled off and the residual liquor poured on to ice. The acid liquors were made alkaline with ammonia and extracted with ether, evaporation of the solvent yielding the ethyl ester (10·1 g.). In a similar way from 368 g. of acid there were obtained 354 g. of ester, b.p. 174–175° at 14 mm.

(*b*) *From aniline*

A practical preparation of cinchoninic acid from aniline was made possible by Michailov's improvements (1936) in the preparation of lepidine from aniline.

Acetoacetanilide. Ethyl acetoacetate (260 g.) was heated in an open flask in an oil bath to 160° and aniline (46·5 g.) was slowly added so that the inside temperature did not fall below 160°. The contents were stirred occasionally to facilitate removal of the ethyl alcohol generated, and heating was continued for 30 min. after all the aniline had been added. On cooling, the anilide crystallized in plates (yield 61·7 g.), which were collected and washed with low boiling petroleum. The filtrate was concentrated under reduced pressure for recovery of excess of ethyl acetoacetate and a second crop of anilide, 17·1 g., collected. The total yield was 89 %. This process is based on that described in brief by Limpach (1931), who records a yield of 90 %.

2-*Chlorolepidine.* 2-Hydroxylepidine was prepared from the above anilide by the process described by Michailov (*loc. cit*). The yield was 80–90 %. From this hydroxy compound 2-chlorolepidine was obtained in 91 % yield by Michailov's method.

Lepidine. This was prepared by Michailov's process by reduction of the chlorolepidine with tin and hydrochloric acid at 70–80° for 10 hr. The crystalline tin salt was collected, dissolved in a large volume of water, decomposed with hydrogen sulphide, the tin sulphides removed and the filtrate concentrated, made alkaline and extracted with ether. The base was then distilled, yield 93 %, b.p. 133·5° at 16 mm. The distillation residues contain small amounts of unchanged hydroxylepidine.

4-*Styrylquinoline.* Lepidine (57·2 g.) was boiled for 5 hr. with benzaldehyde (230 c.c.) and anhydrous zinc chloride (24 g.). When cold the contents of the flask were warmed on the water bath with 2*N*-hydrochloric acid (600 c.c.) and benzene. The precipitated styrylquinoline hydrochloride was collected and washed with benzene. The hydrochloride was decomposed with sodium hydroxide solution (50 %) and the base extracted with ether. On removal of the ether the residue crystallized and for purification was washed with low boiling petroleum, yield 79·5 g. A small quantity of base could be recovered from the petroleum as hydrochloride, bringing the total yield to 90 %. Benzaldehyde was recovered from the benzene fraction by distillation.

Cinchoninic acid. 4-Styrylquinoline (23·1 g.) in 50 % aqueous pyridine solution (500 c.c.) was treated at 0 to 5° with mechanical stirring with finely powdered potassium permanganate (42·0 g.) over a period of 30 min. Stirring was continued for a further 2 hr., the temperature being allowed to rise to room temperature. The manganese oxides were collected and the filtrate, without addition of any washings, was concentrated under reduced pressure to a small volume so as to recover the 50 % pyridine for use in a repetition of the process. The manganese oxides were suspended in water and heated on the water bath and then filtered to recover the remainder of the oxidation products. The filtrate and the original concentrate were combined, reconcentrated to a small volume and acidified to neutrality to Congo-paper. The precipitated acids were collected, washed with water and dried and then shaken with cold ether to remove benzoic acid, and finally dried, yield 98 %. This method is based on an analogous one described by Rabe, Huntenburg, Schultze and Volger (1931). The average yield of cinchoninic acid over many experiments was 225 g. of reprecipitated pure acid from 326 g. of aniline.

(*c*) *From isatin*

The preparation of cinchoninic acid from isatin is probably the most convenient and most economical.

Carbostyril-4-carboxylic acid. N-Acetylisatin (56·7 g.) prepared in 88 %

yield by Camps' process (1899), was boiled for 1 hr. under reflux with sodium hydroxide (30 g.) in water (1800 c.c.). The solution was cooled, neutralized to Congo-paper and the precipitate collected after washing with water. It was treated with saturated sodium bicarbonate solution (360 c.c.), which left isatin (11 g.) undissolved. The filtrate was acidified and gave carbostyril-4-carboxylic acid, yield 39·8 g., m.p. 342°. This is an 85·2 % yield, allowing for recovered isatin. Camps (1899), by a slightly different process, records an 80 % yield.

2-*Chloro*-4-*cinchoninic acid*. The hydroxy acid (18·9 g.) was heated with phosphorus oxychloride (45·9 g.) for 30 min. in an oil bath at 130–140°. After cooling, the dark brown liquid was poured into water, kept for 2 hr. and then the solid collected. It was purified by solution in sodium bicarbonate solution and precipitation by acid, yield 18·7 g. (91·3 %). 2-Chlorocinchoninic acid softens and effervesces at 200° and finally melts at 233–235°.

Cinchoninic acid. 2-Chlorocinchoninic acid (20·7 g.) was dissolved in $2N$-sodium hydroxide solution (100 c.c.) and then diluted with water (500 c.c.). The dissolved acid was then catalytically reduced (Soc. chem. Ind. in Basle, 1936) in presence of palladized charcoal (3 g.). The catalyst was collected and dried for use in subsequent batches. The filtrate was neutralized to Congo-paper and the precipitated cinchoninic acid collected and dried, yield 16·8 g. (97·1 %).

Using the isatin process, 250·7 g. of isatin gave 200 g. of pure cinchoninic acid.

Condensation of ethyl cinchoninate with ethyl ε-benzamidocaproate

A series of methods were explored using a variety of condensing agents in order to determine the optimum conditions for the preparation of α-carbethoxy-ε-benzamidoamyl-4-quinolyl ketone (VII, $R = \mathrm{H}$), and hence of ε-benzamidoamyl-γ-quinolyl ketone. On account of the low yield (17 %) of α-carbethoxy-ε-benzamidoamyl-4-quinolyl ketone obtained when ethyl cinchoninate and ethyl ε-benzamidocaproate were condensed in the presence of sodium ethoxide (Ruzicka, Seidel and Liebl 1924), this method was not repeated. Using sodium wire the yield was raised to 34 % and a similar yield was obtained using powdered sodium. When ethyl cinchoninate was replaced by the methyl ester the yield fell to 25 %. A workable process giving 64 % yield was eventually obtained by use of sodamide.

Several experiments were carried out using commercial sodamide, but although the yield of α-carbethoxy-ε-benzamidoamyl-4-quinolyl ketone was considerably increased in the majority of cases, a variation in the extent of condensation occurred with different batches of sodamide. In some experiments no condensation product was obtained and the esters were recovered unchanged. This was eventually traced to the presence of nitrite in the sodamide used. It was found that nearly all batches of sodamide contained detectable amounts of sodium nitrite, the efficiency of the amide as a condensing agent decreasing rapidly with the increase in the amount of nitrite.

Sodamide (10 g.), free from nitrite, was powdered under dry benzene (20 c.c.) and the suspension added to a solution of ethyl cinchoninate (40·2 g.) and ethyl ε-benzamidocaproate (56·2 g.) in benzene (60 c.c.). On boiling the mixture under reflux, evolution of ammonia began almost immediately and was accompanied by the formation of a white gelatinous precipitate which gradually filled the liquid (1·5 hr.). Further heating caused what appeared to be a second reaction to occur, the pasty mass slowly changing into a heavy viscous dark brown oil with a clear upper benzene layer. After boiling for 7 hr., evolution of ammonia being complete, the mixture was allowed to stand overnight, the benzene decanted and the viscous syrup dissolved in water. Saturation of the aqueous extract with carbon dioxide caused the separation of a heavy viscous syrup, extraction with chloroform and evaporation of the solvent giving 54·8 g. of crude α-carbethoxy-ε-benzamidoamyl-4-quinolyl ketone, yield 64 %.

ε-Benzamidoamyl-4-quinolyl ketone (IX, $R = \mathrm{H}$). α-Carbethoxy-ε-benzamidoamyl-4-quinolyl ketone, as obtained above, was hydrolysed by heating on the water bath with 17 % hydrochloric acid (250 c.c.) for 1·5 hr. The dark brown solution was poured on to ice, made alkaline with potassium carbonate and extracted with chloroform, evaporation of the solvent yielding crude *ε-benzamidoamyl-4-quinolyl ketone* (33·7 g.) which readily crystallized. It was purified by crystallization from acetone, or better, from methyl alcohol, from which solvents it separates in clusters of colourless stout needles, m.p. 113–113·5°. (Found:* C, 76·4; H, 6·5; N, 8·2. $C_{22}H_{22}O_2N_2$ requires C, 76·3; H, 6·4; N, 8·1 %.) Under the hydrolytic conditions described above there is very little fission of the benzamide group.

ε-Aminoamyl-4-quinolyl ketone. Preliminary hydrolytic experiments, with ether extraction of the liberated benzoic acid at 3 hr. intervals, showed that the hydrolysis of the benzamido group of ε-benzamidoamyl-4-quinolyl ketone was very slow, approximately 15 hr. boiling with 20 % hydrochloric acid being necessary to effect complete removal of the benzoyl group. Accordingly ε-benzamidoamyl-4-quinolyl ketone (75 g.) was boiled under reflux with constant boiling hydrochloric acid (400 c.c.) for 15 hr., cooled, poured on to ice and made alkaline with 50 % aqueous sodium hydroxide solution. During liberation of the base, rise of temperature must be avoided, otherwise rapid darkening of the amine occurred. Extraction of the base with chloroform and evaporation of the solvent below 40° gave ε-aminoamyl-4-quinolyl ketone (51 g.) as a pale brown syrup which could not be crystallized.

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* All analyses are "micro".

α-Bromo-ε-aminoamyl-4-quinolyl ketone dihydrobromide (X, $R = H$). ε-Aminoamyl-4-quinolyl ketone (13·5 g.) was dissolved in cold 40 % aqueous hydrobromic acid (43 c.c.) at 0° to avoid excessive darkening of the liquid. The solution was then warmed to 50° and bromine vapour from 8·9 g. of bromine passed into the rapidly stirred solution in a current of nitrogen. Absorption of the bromine was quantitative and was complete in 3·5 hr. After standing at 0° overnight, the dirty-white crystalline solid was filtered and washed free from excess of hydrobromic acid with absolute alcohol. In this way 19·6 g. of pure α-bromo-ε-aminoamyl-4-quinolyl ketone dihydrobromide, m.p. 185–186° (decomp.), was obtained. Ruzicka, Seidel and Liebl (1924), for the recrystallized dihydrobromide, give m.p. 185°. The dark brown mother liquors and washings were evaporated *in vacuo* at 50° and the very dark partly crystalline residue thoroughly washed with absolute alcohol. In this way a further 4·7 g. of dihydrobromide was obtained, making a total yield of 90 %.

4-Quinolyl-α-piperidyl ketone (VIII, $R = H$). Since the method employed by Ruzicka, Seidel and Liebl (1924) for the cyclization of α-bromo-ε-aminoamyl-4-quinolyl ketone dihydrobromide was unsatisfactory, various other methods were tried using weakly alkaline reagents to neutralize the hydrobromic acid and effect ring closure. With this object in view, aqueous ammonia, sodium bicarbonate and sodium carbonate were used. With sodium bicarbonate the time of shaking in presence of ether and alkali proved to be an important factor, shaking for 3 min. causing elimination of 30 %, for 10 min. 70 %, and for 20 min. 76 % of the substituted bromine. Replacement of sodium bicarbonate by 2*N*-ammonia or saturated sodium carbonate solution effected the elimination of over 90 % of the substituted bromine in 20 min.

α-Bromo-ε-aminoamyl-4-quinolyl ketone dihydrobromide (10 g.) was dissolved in water (100 c.c.), saturated sodium carbonate solution (100 c.c.) and ether (150 c.c.) added, and shaken vigorously for 20 min. The alkaline liquors were extracted again with ether (2 × 100 c.c.) and the combined ethereal extracts evaporated under reduced pressure below 20°. An orange-yellow oil of crude 4-quinolyl-α-piperidyl ketone was thus obtained. On account of its tendency to undergo further change with rapid deepening of colour, this ketone proved difficult to characterize. The sole crystalline salt, the *dipicrolonate*, m.p. 181–182° (decomp.), was obtained by addition of a cold ethyl alcoholic solution of the ketone to excess of a warm alcoholic solution of picrolonic acid and allowing the solution to stand for 2 days at 0°. Spherical clusters of orange-brown plates separated which could be recrystallized from the same solvent. (Found: C, 54·4; H, 4·3; N, 18·4. $C_{35}H_{32}O_{11}N_{10}$ requires C, 54·7; H, 4·2; N, 18·2 %.)

4-*Quinolyl-α-piperidylcarbinol and its derivatives*

4-*Quinolyl-α-piperidylcarbinol* (V, $R = H$). For the reduction of 4-quinolyl-α-piperidyl ketone to the carbinol it is always necessary to start from α-bromo-ε-aminoamyl-4-quinolyl ketone dihydrobromide, to cyclize this to the bromine-free ketone as described above and to reduce the crude ketone catalytically without any delay.

Crude 4-quinolyl-α-piperidyl ketone (5·2 g.) from 10 g. of dihydrobromide was dissolved in methyl alcohol (50 c.c.), platinum oxide (0·13 g.) added, and shaken with hydrogen until absorption was complete. (Hydrogen absorbed, 489 c.c.; calc. 485 c.c.) The filtered alcoholic solution on evaporation below 40° left the crude carbinol (5·2 g.) as a pale brown syrup. It was dissolved in methyl alcohol (25 c.c.), concentrated sulphuric acid (1·2 c.c.) added, and the solution kept at 0° overnight. 4-*Quinolyl-α-piperidylcarbinol sesquisulphate* was obtained as a brown crystalline crust (2·15 g.) which crystallized readily from methyl alcohol in colourless prisms, m.p. 172° (decomp.). (Found: C, 45·4; H, 5·8; N, 7·0; SO_4, 36·2. $2C_{15}H_{18}ON_2, 3H_2SO_4, H_2O$ requires C, 45·2; H, 5·5; N, 7·0; SO_4, 36·2 %.) The *normal sulphate* was prepared from the carbinol (0·24 g.) in methyl alcohol (10 c.c.) and sulphuric acid (0·03 c.c.). After addition of an equal volume of acetone, most of the solvent was removed, and on keeping at 0° a crystalline crust was obtained which could be crystallized from aqueous acetone in stout colourless prisms, m.p. 129° (decomp.). (Found: C, 45·9; H, 6·6; SO_4, 24·9. $C_{15}H_{18}ON_2, H_2SO_4, 3H_2O$ requires C, 45·5; H, 6·6; SO_4, 24·8 %.)

It was found subsequently that a more complete separation of 4-quinolyl-α-piperidylcarbinol could be effected by isolation as the dihydrochloride. Thus the bromo-dihydrobromide (20 g.) on cyclization and reduction gave crude 4-quinolyl-α-piperidylcarbinol (6·9 g.). It was dissolved in ethyl alcohol (30 c.c.), ethyl alcoholic hydrogen chloride (5·6 c.c. of 37 % solution) added, and after standing overnight at 0° filtered. 4-*Quinolyl-α-*

piperidylcarbinol dihydrochloride (4·15 g.) was obtained which crystallized from aqueous acetone in long thin colourless prisms, m.p. 230° (decomp.). (Found: C, 54·4; H, 6·2. $C_{15}H_{18}ON_2$, 2HCl, H_2O requires C, 54·1; H, 6·6 %.) This salt is very readily soluble in water. 4-*Quinolyl*-4-*piperidylcarbinol base* regenerated from the pure sesquisulphate through ether, crystallized from dry ether or acetone in colourless prisms, m.p. 143–144°. (Found: C, 74·4; H, 7·4; N, 12·0. $C_{15}H_{18}ON_2$ requires C, 74·4; H, 7·4; N, 11·6 %.)

4-*Quinolyl*-α-*piperidylcarbinol hydrochloride*. The base (1·2 g.) was dissolved in ethyl alcohol (15 c.c.) and ethyl alcoholic hydrogen chloride (0·5 c.c. of 36·5 % solution; 1 mol.) added. Crystallization began immediately and the mixture set to a pale cream magma. The solid (1·2 g.) was collected and crystallized from 95 % alcohol or water, when it separated in rod-like prisms, m.p. 202° (decomp.). (Found: C, 60·5; H, 6·8; H_2O, 6·4. $C_{15}H_{18}ON_2$, HCl, H_2O requires C, 60·7; H, 7·1; H_2O, 6·1 %.) The aqueous solution had a neutral reaction, *p*H 6·5–7.

4-*Quinolyl*-α-N-*nitrosopiperidylcarbinol*. The carbinol dihydrochloride (0·1 g.) was dissolved in water (2 c.c.) and aqueous sodium nitrite (0·12 c.c. of a 30 % solution) added. The solution became pale yellow and after 2 days deposited clusters of colourless crystals. 4-*Quinolyl*-α-N-*nitrosopiperidylcarbinol* is almost insoluble in water but crystallizes readily from aqueous alcohol in colourless hexagonal plates, m.p. 189° (decomp.), giving a strong Liebermann reaction. (Found: C, 66·2; H, 6·3. $C_{15}H_{17}O_2N_3$ requires C, 66·4; H, 6·3 %.)

4-*Quinolyl*-α-N-*methylpiperidylcarbinol methiodide* (XIII). The parent crude carbinol (0·7 g.) was dissolved in acetone (5 c.c.) and methyl iodide (0·22 c.c.; 1·3 mol.) added. There was a slight evolution of heat and the liquid darkened gradually to red-brown. On keeping for 12 hr. a brown syrup separated which, on rubbing, crystallized to a pale brown powder (0·36 g.). Repeated crystallization from water gave 4-*quinolyl*-α-N-*methylpiperidylcarbinol methiodide* as long colourless prisms, m.p. 216° (decomp.). (Found: C, 49·0; H, 5·8; N, 6·9. $C_{17}H_{23}ON_2I$, H_2O requires C, 49·0; H, 6·0; N, 6·7 %.) This mono-quaternary salt also occurs in another form, m.p. 226–227°, a mixture of the two forms showing an intermediate melting point. (Found for higher melting form: C, 49·3; H, 6·1; N, 7·2 %.) The aqueous crystallization mother liquors from a larger scale experiment using 3·0 g. of carbinol were made alkaline with sodium carbonate and extracted with chloroform, yielding 0·7 g. of a pale brown syrup. This was dissolved in acetone (5 c.c.), *N*-hydrochloric acid (2·75 c.c.) added and finally sufficient acetone (16 c.c.) to produce a faint turbidity. On keeping below 0° for 3 days, colourless prisms (0·45 g.) of 4-*quinolyl*-α-N-*methylpi*-

peridylcarbinol hydrochloride, m.p. 138° (decomp.), separated. It was unchanged in melting point on recrystallization from aqueous acetone. (Found: C, 61·6; H, 7·5. $C_{16}H_{20}ON_2$, HCl, H_2O requires C, 61·8; H, 7·5 %.) The original acetone reaction mother liquors were treated with aqueous sodium carbonate and extracted with chloroform, evaporation of the solvent leaving a gum (1·85 g.) which, on keeping in acetone (10 c.c.) below 0° for several days, gave unchanged 4-quinolyl-α-piperidylcarbinol (0·4 g.), m.p. 139–140°.

If methylation of 4-quinolyl-α-piperidylcarbinol is carried out in the presence of anhydrous sodium or potassium carbonate or sodium bicarbonate using two or three molecular proportions of methyl iodide, 4-quinolyl-α-*N*-methylpiperidylcarbinol methiodide is the predominant product.

4-*Quinolyl-α-*N*-methylpiperidylcarbinol dimethiodide*. Crude 4-quinolyl-α-piperidylcarbinol (1·0 g.) in methyl alcohol (10 c.c.) was boiled with methyl iodide (2·5 c.c.; 10 mol.) for 4·5 hr. A crystalline product gradually separated from the hot solution and increased in quantity with time. When cold the solid (0·82 g.) was collected and crystallized from water, from which it separated in bright orange tablets, m.p. 244° (decomp.). (Found: C, 39·0; H, 4·8. $C_{18}H_{26}ON_2I_2.H_2O$ requires C, 38·7; H, 5·0 %.)

4-*Quinolyl-α-*N*-methylpiperidylcarbinol methochloride*. 4-Quinolyl-α-*N*-methylpiperidylcarbinol methiodide (2·0 g.) in 115 c.c. of 80 % alcohol was shaken with excess of freshly prepared silver chloride for 30 min. The filtered solution was rapidly concentrated under reduced pressure to a small volume. The *methochloride* which separated (1·0 g.) was crystallized from water in which it is moderately soluble and separated in clusters of colourless prisms, m.p. 168–169° (decomp.). (Found: C, 55·2; H, 8·0. $C_{17}H_{28}ON_2Cl$, $3\frac{1}{2}H_2O$ requires C, 55·2; H, 8·2 %.)

4-*Quinolyl-α-*N*-methylpiperidylcarbinol*. The mono-methiodide (2·5 g., m.p. 216°) was converted into the methochloride in solution as described above and then into the quaternary hydroxide by shaking the solution with excess of freshly precipitated silver oxide for 30 min. The filtrate was saturated with hydrogen sulphide, then evaporated to dryness at 50° and the evaporation repeated in presence of ethyl alcohol. The flask was then immersed in a boiling water bath and the contents (1·67 g.) heated in the vacuum of a water pump until the evolution of methyl sulphide was complete (45 min.). The ether-soluble material (1·12 g.), a pale golden-brown syrup, slowly crystallized when kept at 35°. 4-*Quinolyl-α-*N*-methylpiperidylcarbinol* crystallized from ether in well-formed colourless hexagonal tablets, m.p. 120–121°. (Found: C, 75·3; H, 7·7; N, 11·0. $C_{16}H_{20}ON_2$ requires C, 75·0; H, 7·9; N, 11·0 %.) On methylation with one molecular proportion of methyl iodide in boiling methyl alcohol for 1 hr., it gave 4-quinolyl-α-*N*-methylpiperidylcarbinol methiodide, m.p. 216°, identical with that previously described.

When this experiment was repeated starting from the higher melting methiodide, m.p. 226–227°, and using acetone for extracting the tertiary base after heat decomposition, a higher melting form of 4-quinolyl-α-*N*-methylpiperidylcarbinol, m.p. 152°, was obtained. It was best recrystallized from ether and showed no depression of melting point when mixed with the form melting at 120–121°. (Found: C, 75·4; H, 8·0; N, 11·3; Calc. C, 75·0; H, 7·9; N, 11·0%.) On remethylation it gave the same quaternary iodide as the starting material, m.p. 226–227°.

*4-Quinolyl-α-*N*-methylpiperidylcarbinol dipicrolonate.* The pure *N*-methyl carbinol (0·1 g.) was dissolved in hot ethyl alcohol (10 c.c.) and added to a boiling solution of picrolonic acid (0·31 g.; 3 mol.) in ethyl alcohol (25 c.c.). In a few minutes separation of a bright yellow crystalline solid (0·24 g.) in the form of leaflets commenced. It was crystallized from boiling ethyl alcohol in which it is sparingly soluble, m.p. 228° (decomp.). (Found: C, 55·2; H, 5·1. $C_{36}H_{36}O_{11}N_{10}$ requires C, 55·1; H, 4·6%.)

Direct mono-methylation of 4-quinolyl-α-piperidylcarbinol. 4-Quinolyl-α-piperidylcarbinol (4·0 g.; 2 mol.) was dissolved in acetone (400 c.c.), methyl iodide (0·51 c.c.; 1 mol.) added, and the solution stirred for 6·5 hr. at the ordinary temperature. Evaporation of the acetone under reduced pressure left a pale brown solid which on treatment with chloroform gave *4-quinolyl-α-piperidylcarbinol hydriodide* (3·1 g.) which crystallized from water in tablets, m.p. 160–161° (decomp.). (Found: C, 46·7; H, 5·2. $C_{15}H_{19}ON_2I$, H_2O requires C, 46·4; H, 5·4%.) Evaporation of the chloroform filtrate gave a syrup (2·55 g.) which on solution in a small volume of ether gave 4-quinolyl-α-*N*-methylpiperidylcarbinol, m.p. 152°, yield 1·7 g.

Attempts to prepare a mono-ethyl derivative of 4-quinolyl-α-piperidylcarbinol by the action of ethyl iodide or bromide were unsuccessful. A by-product in the action of ethyl bromide was *4-quinolyl-α-piperidylcarbinol hydrobromide*, m.p. 196–197° (decomp.), which crystallized from water in long thin rhomboidal plates. (Found: C, 52·8; H, 6·1. $C_{15}H_{19}ON_2$, HBr, H_2O requires C, 52·8; H, 6·2%.) Its constitution was proved by regeneration to the base.

*4-Quinolyl-α-*N*-ethylpiperidylcarbinol methiodide* (XIV, *R* = Et). 4-Quinolyl-α-*N*-methylpiperidylcarbinol (0·5 g.) was dissolved in acetone (25 c.c.), ethyl iodide (0·32 c.c.; 2 mol.) added, and the solution kept at 37° for 18 days. After removal of the solvent at low temperature, the residue when dissolved in a small volume of water gave the quaternary salt (0·15 g.) in colourless short stout prisms. On crystallization from water it gave a polyhydrate with an indefinite melting point (114–115°, resolidifying and then decomposing at 155° with previous softening). Specimens dried at 95° showed a constant decomposition point of 162–163°. (Found on material dried at 95°: C, 47·9; H, 6·5. $C_{18}H_{25}ON_2I, 2H_2O$ requires C, 48·2; H, 6·5 %.)

4 - *Quinolyl* - *α* - N - *allylpiperidylcarbinol methiodide* (XIV, $R = .CH_2.CH{:}CH_2$). 4-Quinolyl-α-*N*-methylpiperidylcarbinol (0·5 g.) was dissolved in methyl alcohol (5 c.c.), allyl iodide (0·35 c.c.; 2 mol.) added, and the solution boiled under reflux for 2 hr. On complete removal of the solvent and solution of the residue in hot water (7 c.c.) an oil separated on cooling which solidified at 0° and on crystallization from water gave colourless thin rhomboidal plates (0·15 g.) of the above-named *methiodide*, m.p. 186–186·5° (decomp.). (Found: C, 51·8; H, 6·3; N, 6·3. $C_{19}H_{25}ON_2I, H_2O$ requires C, 51·6; H, 6·2; N, 6·3 %.)

Concentration of the mother liquors and crystallization of the product from water gave lemon-yellow needles of 4-*quinolyl*-*α*-N-*methylpiperidylcarbinol diallyl iodide*, m.p. 160–161°, in very small yield. (Found: C, 42·9; H, 5·5; N, 4·4. $C_{22}H_{30}ON_2I_2, 1{\cdot}5H_2O$ requires C, 42·7; H, 5·4; N, 4·5 %.)

4 - *Quinolyl* - *α* - N - *crotylpiperidylcarbinol methiodide* (XIV, $R = .CH_2.CH{:}CH.CH_3$). Crotyl bromide (0·41 c.c.; 2 mol.) was added to a solution of sodium iodide (0·4 g.; 2 mol.) in acetone (10 c.c.), filtered from precipitated sodium bromide and the filtrate added to a solution of 4-quinolyl-α-*N*-methylpiperidylcarbinol (0·5 g.; 1 mol.) in acetone (10 c.c.), and the mixture boiled for 1 hr. On removal of the solvent and on solution of the residue in a small volume of water, a crystalline solid was obtained which on repeated crystallization from water to constant melting point gave 4-quinolyl-α-*N*-crotylpiperidylcarbinol methiodide, m.p. 174–175° (decomp.), as colourless rectangular tablets. (Found: C, 52·0; H, 6·3; N, 6·3. $C_{20}H_{27}ON_2I, H_2O$ requires C, 52·6; H, 6·4; N, 6·1 %.)

4-*Quinolyl*-*α*-N-*allylpiperidylcarbinol hydriodide*. Allyl iodide prepared from allyl bromide (0·36 c.c.) and sodium iodide (0·62 g.) in acetone (10 c.c.) was added to a solution of 4-quinolyl-α-piperidylcarbinol (1·0 g.) in methyl alcohol (5 c.c.) and boiled gently under reflux for 4 hr. On complete removal of the solvents and solution of the residual syrup in acetone and keeping overnight at −4°, a crystalline crop (0·4 g.) was obtained which on fractional crystallization from water gave 4-quinolyl-α-piperidylcarbinol hydriodide, m.p. 160–161°, and 4-*quinolyl*-*α*-N-*allylpiperidylcarbinol hydriodide* as large colourless elongated tablets, m.p. 190–191° (decomp.), yield 0·1 g. (Found: C, 50·7; H, 5·9. $C_{18}H_{23}ON_2I, H_2O$ requires C, 50·5; H, 5·9 %.) The acetone mother liquors of the hydriodides contained a syrup which on conversion into base and then into the dihydrochloride in alcoholic solution gave a small amount of unchanged 4-quinolyl-α-piperidylcarbinol dihydrochloride (0·1 g.), m.p. 223–224° (decomp.).

An improvement on the above process for preparing the *N*-allyl derivative was effected by carrying out the reaction at room temperature in presence of sodium bicarbonate. A solution of allyl iodide, from allyl bromide (1·46 g.; 1·25 mol.) and sodium iodide (2·5 g.; 1·25 mol.) in acetone (100 c.c.), was added to a suspension of sodium bicarbonate (3·0 g.) in a solution of 4-quinolyl-α-piperidylcarbinol (3·25 g.) in acetone (250 c.c.) and stirred vigorously for 6 hr. at room temperature. The solid phase (4·9 g.) was collected, dissolved in water (50 c.c.) and kept for 3 hr. The precipitated base was extracted with light petroleum, giving 0·8 g. of unchanged 4-quinolyl-α-piperidylcarbinol, m.p. 141–142°.

The acetone filtrate was evaporated dry under reduced pressure and the residual syrup (3·9 g.) dissolved in ethyl alcohol (15 c.c.), cooled to 10°, ethyl alcoholic hydrogen chloride (3·3 c.c. of a 35 % solution) added, and after standing at 0° for 2 hr., filtered. *4-Quinolyl-α-*N*-allylpiperidylcarbinol trihydrochloride* (2·45 g.) was thus obtained which crystallized from ethyl alcohol in thin colourless plates, which darkened at 153° and decomposed slowly between 180 and 190°. (Found: C, 50·7, 50·5; H, 6·7, 6·4. $C_{18}H_{22}ON_2$, 3HCl, $2H_2O$ requires C, 50·5; H, 6·8 %.) Regeneration of the *base* from the trihydrochloride gave a syrup, 1·25 g., which crystallized from light petroleum in rosettes of colourless prisms, yielding 1·0 g., m.p. 86°. (Found: C, 76·6; H, 7·8. $C_{18}H_{22}ON_2$ requires C, 76·6; H, 7·9 %.)

*4-Quinolyl-α-*N*-allylpiperidylcarbinol hydrochloride.* The base (0·37 g.) was dissolved in one equivalent of deci-normal hydrochloric acid and the aqueous solution concentrated. The *monohydrochloride* crystallized from a small volume of water in needles, m.p. 87°. (Found: C, 60·8; H, 7·7. $C_{18}H_{22}ON_2$, HCl, $2H_2O$ requires C, 60·9; H, 7·7 %.)

*4-Quinolyl-α-*N*-propylpiperidylcarbinol.* 4-Quinolyl-α-*N*-allylpiperidylcarbinol (3·5 g.) was dissolved in methyl alcohol (35 c.c.), platinum oxide (0·16 g.) added, and shaken in an atmosphere of hydrogen until absorption was complete. (Hydrogen absorption found = 335 c.c.; calc. for one double bond and catalyst, 307 c.c.). The black colloidal solution was evaporated dry at low temperature and the residue extracted with ether, which caused the platinum to coagulate. The ethereal filtrate concentrated to 20 c.c. and kept at 0° overnight gave 4-quinolyl-α-piperidylcarbinol (1·2 g.), m.p. 139–140°, as a crystalline crust. The ethereal mother liquors were concentrated to 5 c.c. and kept overnight below 0°, when 1·15 g. of 4-

*quinolyl-α-*N*-propylpiperidylcarbinol* were obtained. This base crystallized from 50 % alcohol in colourless hexagonal prisms, m.p. 100–101°. (Found: C, 76·3; H, 8·6. $C_{18}H_{24}ON_2$ requires C, 76·1; H, 8·5 %.) The *dihydrochloride* was prepared by the addition of two molecular proportions of ethyl alcoholic hydrogen chloride to an alcoholic solution of the propyl base. It crystallized from alcohol in hexagonal plates, m.p. 139–140° (decomp.). (Found: C, 52·6; H, 7·6. $C_{18}H_{24}ON_2$, 2HCl, $3H_2O$ requires C, 52·5; H, 7·8 %.) This salt is very soluble in water. The *monohydrochloride* was prepared by dissolving the base (1·15 g.) in hot aqueous *N*-hydrochloric acid (4·1 c.c.). It crystallized in diamond-shaped plates (1·0 g.), m.p. 69–70°, which have a neutral reaction in solution. (Found: C, 55·3; H, 8·6; N, 7·5. $C_{18}H_{24}ON_2$, HCl, $4H_2O$ requires C, 55·0; H, 8·5; N, 7·1 %.)

4-*Quinolyl-α-*N*-crotylpiperidylcarbinol*. 4-Quinolyl-α-piperidylcarbinol (5·0 g.) was dissolved in acetone (400 c.c.), a solution of crotyl iodide prepared from crotyl bromide (2·5 c.c.) and sodium iodide (3·9 g.) in acetone (100 c.c.) and sodium bicarbonate (5·0 g.) added and stirred at room temperature for 6 hr. After standing overnight, the unchanged sodium bicarbonate was removed and the acetone evaporated dry. The residual brown syrup was dissolved in alcohol and treated with ethyl alcoholic hydrogen chloride (7·5 c.c. of a 37 % solution), when a crude dihydrochloride (7·8 g.), m.p. 195–196° (decomp.), was precipitated as a microcrystalline powder. This solid was regenerated to base in ethereal solution and on concentration of the solvent there separated in succession 4-quinolyl-α-piperidylcarbinol (0·7 g., m.p. 140–141°) and the much more soluble 4-*quinolyl-α-*N*-crotylpiperidylcarbinol*, m.p. 89–90°. The latter crystallized from ether in bold colourless rectangular prisms. (Found: C, 77·6; H, 8·4; N, 9·6. $C_{19}H_{24}ON_2$ requires C, 77·0; H, 8·2; N, 9·5 %.) By solution of the crotyl base in one equivalent of *N*-hydrochloric acid and concentration over sulphuric acid, compact clusters of colourless tablets of 4-*quinolyl-α-*N*-crotylpiperidylcarbinol monohydrochloride*, m.p. 86–87°, were obtained. (Found: C, 61·6; H, 7·9; N, 7·0. $C_{19}H_{24}ON_2$, HCl, $2H_2O$ requires C, 61·8; H, 7·9; N, 7·6 %.)

4-*Quinolyl-α-*N*-butylpiperidylcarbinol* (VI, $R = H$). Attempts to prepare this base by direct alkylation were all unsuccessful. It was however obtained from the crotyl base as follows. 4-Quinolyl-α-*N*-crotylpiperidylcarbinol (1·6 g.) was dissolved in methyl alcohol (30 c.c.), platinum oxide (0·124 g.) added and shaken in hydrogen. Hydrogen absorption proceeded smoothly until 1·3 mol. of hydrogen had been taken up and then a sudden increase in the rate of absorption occurred. The reaction was interrupted at this stage when 187 c.c. of hydrogen had been absorbed. (Calc. for one double bond and catalyst 144 c.c.). The solution was filtered from the catalyst and evaporated to a syrup which on solution in ether (20 c.c.) and keeping at 0° gave 4-quinolyl-α-piperidylcarbinol (0·83 g.), m.p. 142–143°. As the butyl base in the ethereal mother liquor could not be crystallized, it was dissolved in $N/5$-hydrochloric acid (8·4 c.c.; 1 equiv.) and the solution concentrated over sulphuric acid. *4-Quinolyl-α-*N*-butylpiperidylcarbinol monohydrochloride* (0·6 g.) crystallized from water in hexagonal tablets, m.p. 100–101°. (Found: C, 61·1; H, 8·4; N, 7·7. $C_{19}H_{26}ON_2$, HCl, $2H_2O$ requires C, 61·5; H, 8·4; N, 7·6 %.) This salt had a neutral reaction and was very soluble in water.

6-*Methoxyquinoline and derived substances*

For the preparation of 6-methoxyquinoline-4-carboxylic acid (quininic acid) in quantity, 6-methoxyquinoline or *p*-anisidine were the starting points, since 6-methoxyisatin is not readily accessible. 6-Methoxyquinoline was obtained in 66 % yield by Skraup's method (1885). It was converted quantitatively into the methosulphate by the method of Kaufmann and Peyer (1912) and then into 6-methoxy-4-cyanoquinolane in 84 % yield by Kaufmann's process (1918). This quinolane gave 71 % of crude 6-methoxy-4-cyanoquinoline methiodide which on purification was reduced to a 41 % yield (Kaufmann, without giving details, records a yield of 74 %). Methyl iodide was split off from the methiodide in boiling ethyl benzoate solution and the methoxycyanoquinoline was extracted from the ethyl benzoate solution diluted with ether with $3N$-hydrochloric acid. The base was then liberated with ammonia, yield 90 %.

Hydrolysis of 6-*methoxy*-4-*cyanoquinoline*. This base (32·8 g.) was boiled for 1·75 hr. with 64 c.c. of 60 % sulphuric acid. The solution was diluted, neutralized to Congo-paper with ammonia and the precipitated crude quininic acid, m.p. 272–275° (yield 94 %), collected.

Esterification. The crude acid (100 g.) was refluxed for 5 hr. with a mixture of absolute alcohol (500 c.c.) and sulphuric acid (80 c.c.). The greater part of the alcohol was then distilled, the residue diluted with ice and water and made alkaline with ammonia. Ethyl quininate was precipitated as a dirty white solid with a reddish tinge, yield 115·6 g. It was distilled *in vacuo* and gave a pale yellow distillate, b.p. 172° at 1 mm., which readily solidified. The yield of pure ester was 93·5 g.

The weakest link in the above process from methoxyquinoline to ethyl quininate was the iodine oxidation of the quinolane. To avoid this a process was worked out from *p*-anisidine *via* *p*-acetoacetanisidide.

p-*Acetoacetanisidide.* Ethyl acetoacetate (203 c.c.) was heated to 160–165° (oil bath 180°) and powdered *p*-anisidine (49·2 g.) added in small amounts at a time during 20 min., care being taken to keep the temperature of the reaction between the above limits. The alcohol formed during the reaction boiled off rapidly and the reaction was complete in a further 10 min. On cooling, *p*-acetoacetanisidide crystallized in colourless plates, m.p. 118–119° (60·6 g.), evaporation of the mother liquor with recovery of solvent yielding a further 7·5 g., total yield 81 %. Limpach (1931), without giving details, claims a 90 % yield.

2-*Hydroxy*-4-*methyl*-6-*methoxyquinoline.* *p*-Acetoacetanisidide (50 g.) was treated at 0° with sulphuric acid (27·5 c.c.) and the mixture gently heated on the water bath, care being taken that the temperature of the reaction mixture did not exceed 100°. After the initial exothermic reaction was complete, the viscous solution was maintained at 95° for 2 hr. 2-Hydroxy-4-methyl-6-methoxyquinoline was precipitated by pouring the hot acid solution into water (500 c.c.), filtered, washed free from acid and dried, yield 36·5 g., 80 %. This product contains traces of unchanged *p*-acetoacetanisidide and is best purified by crystallization from glacial acetic acid, from which it separates in stout prisms, m.p. 268–270° (decomp.). Rabe, Huntenburg, Schultze and Volger (1931), give m.p. 253°.

2-*Chloro*-4-*methyl*-6-*methoxyquinoline.* The above carbostyril (50 g.) and phosphorus oxychloride (150 c.c.) were heated at 130–140° (bath temperature) for 45 min. It was necessary to remove the bath during the initial vigorous reaction, but after the evolution of hydrogen chloride had moderated, heating was continued. Complete solution occurred in 10–15 min. and then separation of 2-chloro-4-methyl-6-methoxyquinoline commenced, the reaction mixture becoming a mass of long thin prisms. After heating for a further 30 min., the greater part of the excess phosphorus oxychloride was distilled *in vacuo* for recovery and the residue stirred well with water (500 c.c.). The white crystalline precipitate was almost pure 2-chloro-4-methyl-6-methoxyquinoline, m.p. 142–144°, yield 98·5 %.

4-*Methyl*-6-*methoxyquinoline.* 2-Chloro-4-methyl-6-methoxyquinoline (40 g.) and anhydrous sodium acetate (16 g.) were dissolved in hot glacial acetic acid (400 c.c.), 3 g. of palladized charcoal added and the whole shaken in an atmosphere of hydrogen, the temperature of the solution being maintained at about 70°. Hydrogen absorption was rapid during the early stages and then gradually slackened until about 90 % of the calculated volume of hydrogen had been absorbed, the final 10 % of hydrogen being taken up very slowly. Reduction was complete in about 4 hr. The hot acetic acid solution was filtered from catalyst, evaporated to a small volume under reduced pressure, diluted with water, basified and extracted with ether. Evaporation of the ether left a 97·5 % yield of crude 4-methyl-6-methoxyquinoline as a pale brown crystalline mass. It was used without further treatment in the next process.

4-*Styryl*-6-*methoxyquinoline*. Prepared substantially by the method of Rabe, Huntenburg, Schultze and Volger (1931), 50 g. of 4-methyl-6-methoxyquinoline gave 87 g. of the sulphate of the styrylquinoline which in its turn gave 61 g. of 4-styryl-6-methoxyquinoline base.

Quininic acid. This preparation is based on that of Rabe, Huntenburg, Schultze and Volger (1931), but modified so as to deal with larger quantities of material. 4-Styryl-6-methoxyquinoline (35 g.) was dissolved in 800 c.c. of 50 % aqueous pyridine, coôled below 0°, stirred vigorously and powdered potassium permanganate (45 g.) added in small amounts at a time during three-quarters of an hour. The temperature was kept below 10° and the stirring continued for 1 hr. The precipitated manganese oxides were filtered dry without washing and the filtrate distilled under reduced pressure so as to recover the 50 % pyridine for use in subsequent batches. The manganese oxides were then washed with hot water and the washings added to the concentrated mother liquors and evaporated to a small volume *in vacuo* at 50°. The concentrate was neutralized to Congo-paper with hydrochloric acid, cooled and filtered. The precipitated solid was freed from benzoic acid by washing thoroughly with alcohol and ether. The yield of quininic acid was 23 g., and although slightly discoloured had the correct m.p. 280°. It was sufficiently pure for conversion to the ethyl ester as previously described.

Condensation of ethyl quininate with ethyl ϵ-benzamidocaproate

α-Carbethoxy-ϵ-benzamidoamyl-4-(6-methoxyquinolyl) ketone (VII, R = OMe). Sodamide (20 g.) was powdered under benzene (20 c.c.) and added to a solution of ethyl quininate (94 g.) and ethyl ϵ-benzamidocaproate (106 g.) in benzene (120 c.c.). The reaction mixture was boiled gently under reflux on the water bath, whereupon there was a vigorous evolution of ammonia and the liquid became pasty due to the separation of a pale brown solid which gradually increased in amount during the first 2 hr. heating. On further boiling the suspended solid slowly changed into a heavy dark brown viscous syrup, insoluble in benzene. Condensation was complete after 7 hr. when ammonia evolution ceased. After standing overnight the extremely viscous syrup was dissolved in ice-water, separated from the benzene and the aqueous solution saturated with carbon dioxide. A pale brown oil was first deposited, followed by a small quantity

of a pale yellow flocculent precipitate as saturation was reduced. Extraction with chloroform left the flocculent precipitate undissolved and after filtration and evaporation of the solvent there were obtained 65·0 g. of α-carbethoxy-ϵ-benzamidoamyl-4-(6-methoxyquinolyl) ketone as a dark brown viscous syrup. This ester gave a brown-violet coloration in alcoholic solution, but the colour was much less intense than that given by the compound without the methoxy group.

When the benzene was replaced by toluene in the above condensation and the temperature of the reaction raised by heating in an oil bath at 120°, the yield of syrup precipitable by carbon dioxide was increased to 55 %. The product did not however behave normally in the next stage of the process, so that toluene as a solvent was abandoned.

ϵ-Benzamidoamyl-4-(6-methoxyquinolyl) ketone (IX, $R = \text{OMe}$). α-Carbethoxy-ϵ-benzamidoamyl-4-(6-methoxyquinolyl) ketone (65 g.) was dissolved in concentrated hydrochloric acid (150 c.c. d. 1·7), water (150 c.c.) added and the turbid liquid heated on the boiling water bath. The dark brown solution slowly evolved carbon dioxide, 2 hr. being necessary for hydrolysis and decarboxylation. The acid solution was poured on ice (200 g.), made alkaline with potassium carbonate and the precipitated dark brown viscous syrup extracted with chloroform. Evaporation of the solvent gave crude ϵ-benzamidoamyl-4-(6-methoxyquinolyl) ketone (46·1 g.) as a very viscous syrup.

Purification was most easily effected through the picrate. The ketone (46·1 g.) was dissolved in alcohol (300 c.c.) and the solution added to a boiling solution of picric acid (30 g.) in alcohol (400 c.c.). On allowing to cool a mixture of pale yellow plates and a brown crystalline crust separated. Repeated crystallization of the more soluble portion of the picrates from alcohol gave a *picrate* in bright yellow elongated hexagonal tablets, m.p. 101–102°. (Found: C, 56·4; 56·2; H, 4·6, 4·5. $C_{23}H_{24}O_3N_2$, $C_6H_3O_7N_3$, $0{\cdot}5H_2O$ requires C, 56·6; H, 4·6 %.) The less soluble portion of the crude picrate was very sparingly soluble in boiling ethyl alcohol but crystallized more readily from butyl alcohol in canary-yellow hexagonal plates, m.p. 156°. (Found: C, 56·9; H, 4·5; N, 11·7. $C_{23}H_{24}O_3N_2$, $C_6H_3O_7N_3$ requires C, 57·5; H, 4·5; N, 11·6 %.)

Regeneration of *ϵ-benzamidoamyl-4-(6-methoxyquinolyl) ketone* from either picrate gave an almost colourless syrup which crystallized readily on triturating with ether. It crystallized readily from 50 % ethyl alcohol in long thin prisms, m.p. 92°. (Found: C, 73·3; H, 6·3; N, 7·1. $C_{23}H_{24}O_3N_2$ requires C, 73·4; H, 6·4; N, 7·4 %.) The yield of recovered crystalline ketone was 34·6 g.

ε-Aminoamyl-4-(6-methoxyquinolyl) ketone. ε-Benzamidoamyl-4-(6-methoxyquinolyl) ketone (34·6 g.) was dissolved in hydrochloric acid (150 c.c. of 20 % solution) and boiled under reflux for 15 hr. The acid solution was poured on to ice, made alkaline with 50 % aqueous sodium hydroxide and the pale brown oil extracted with chloroform. Evaporation of the chloroform, below 40°, gave ε-aminoamyl-4-(6-methoxyquinolyl) ketone as a brown syrup (20·6 g.).

α-Bromo-ε-aminoamyl-4-(6-methoxyquinolyl) ketone dihydrobromide (X, $R = \text{OMe}$). The crude aminoketone (20·6 g.), obtained as above described, was straightway dissolved in 40 % aqueous hydrobromic acid (63 c.c.) at 0°. The solution was then warmed to 50° and bromine vapour from 4·2 c.c. of bromine passed into the vigorously stirred liquid in a stream of nitrogen during 3 hr. Absorption of bromine was instantaneous and complete. The solution kept overnight at 0° gave a pale yellow crystalline product (26·5 g.) which was well washed with absolute alcohol. The mother liquors on concentration gave a further 7·2 g. of product, bringing the total yield to 87 %. *α-Bromo-ε-aminoamyl-4-(6-methoxyquinolyl) ketone dihydrobromide* crystallized readily from 40 % hydrobromic acid in pale yellowish-buff rhomboidal prisms, m.p. 195° (decomp.). (Found: C, 37·7; H, 4·3; N, 5·5. $C_{16}H_{19}ON_2Br$, 2HBr requires C, 37·4; H, 4·1; N, 5·5 %.)

4-(6-*Methoxyquinolyl*)-*α-piperidylcarbinol* (V, $R = \text{OMe}$). The previously described bromoamine dihydrobromide (20 g.) was dissolved in water (300 c.c.), ether (250 c.c.) and saturated aqueous sodium carbonate solution (150 c.c.) added, and shaken continuously for 20 min. The pale yellow ethereal solution was separated and the aqueous alkaline liquors again extracted with ether (2 × 100 c.c.). The combined ethereal extracts were evaporated to dryness under reduced pressure at the room temperature, crude 4-(6-methoxyquinolyl)-α-piperidylcarbinol ketone being obtained as a brown syrup. This was straightway dissolved in methyl alcohol (100 c.c.), platinum oxide (0·114 g.) added and the solution shaken in an atmosphere of hydrogen until no further absorption occurred. (Hydrogen absorbed 885 c.c., calculated for one ketone group and catalyst 859 c.c.) Filtration and evaporation of the solvent under reduced pressure gave a viscous greenish-brown syrup (10·1 g.). It crystallized on treatment with acetone and on crystallization from the same solvent to constant melting point gave 4-(*6-methoxyquinolyl*)-*α-piperidylcarbinol* (2·4 g.) prisms, m.p. 162–163°. (Found: C, 70·6; H, 7·4; N, 10·6. $C_{16}H_{20}O_2N_2$ requires C, 70·5; H, 7·4; N, 10·3 %.) The combined acetone mother liquors were evaporated dry, the residual syrup (5·7 g.) dissolved in ethyl alcohol and ethyl alcoholic hydrogen chloride (4·2 c.c. of a 37 % solution) added. The solution darkened considerably and slowly deposited 4-(6-methoxyquinolyl)-α-piperidylcarbinol dihydrochloride (1·75 g.), m.p. 231–232° (decomp.). The *monohydrochloride* was obtained from equivalent proportions of the components in aqueous solution. It is readily soluble in warm water and crystallizes in small square plates, m.p. 221° (decomp.). (Found: C, 61·8; H, 6·9; N, 9·0. $C_{16}H_{20}O_2N_2$, HCl requires C, 62·2; H, 6·9; N, 9·1 %.)

iso-4-(6-*Methoxyquinolyl)-α-piperidylcarbinol*. In one instance the stereoisomeric *iso*-base was isolated. The bromoamine dihydrobromide (20 g.) was cyclized and reduced as described above. The base was dissolved in acetone and gave 2·5 g. of 4-(6-methoxyquinolyl)-α-piperidylcarbinol, m.p. 161–162°. The filtrate on keeping for some time gave a second crop of crystals, 1·45 g., of the *iso*-base, which crystallized from acetone in elongated hexagonal prisms, m.p. 187–188°. (Found: C, 70·4; H, 7·4; N, 10·5. $C_{16}H_{20}O_2N_2$ requires C, 70·5; H, 7·4; N, 10·3 %.) The combined mother liquors then gave a residual syrup, which treated with excess of alcoholic hydrogen chloride yielded 1·6 g. of 4-(6-methoxyquinolyl)-α-piperidylcarbinol dihydrochloride, m.p. 231–232° (decomp.).

iso-4-(6-*Methoxyquinolyl)-α-piperidylcarbinol hydrochloride*. The *iso*-base (0·5 g.) was dissolved in warm fifth-normal hydrochloric acid (9·2 c.c.) and the solution cooled to 0° overnight. iso-4-(6-*methoxyquinolyl)-α-piperidylcarbinol hydrochloride* (0·35 g.) separated in hexagonal tablets, m.p. 206–207° (decomp.). (Found: C, 58·9; H, 7·2; N, 8·8. $C_{16}H_{20}O_2N_2$, HCl, H_2O requires C, 58·8; H, 7·1; N, 8·6 %.) In aqueous solution this salt has a neutral reaction.

4-(6-*Methoxyquinolyl)-α-*N-*methylpiperidylcarbinol methiodide*. The carbinol base (2·0 g.) was dissolved in methyl alcohol (25 c.c.), sodium bicarbonate (1·5 g.) and methyl iodide (1·85 c.c.) added, and boiled for 20 min. By this time evolution of carbon dioxide had ceased and the quaternary *methiodide* had separated as a mass of pale cream needles (2·3 g.) which filled the liquid. This salt crystallizes from water in pale orange-yellow hexagonal prisms or pale yellow needles, both with the same m.p. 251° (decomp.). (Found: C, 50·8; H, 5·7; N, 6·5. $C_{18}H_{25}O_2N_2I$ requires C, 50·5; H, 5·9; N, 6·5 %.)

4-(6-*Methoxyquinolyl)-α-*N-*methylpiperidylcarbinol*. Attempts to prepare this carbinol by the action of heat on the quaternary methosulphide were unsuccessful. It was however obtained by direct methylation of the parent base. 4-(6-Methoxyquinolyl)-α-piperidylcarbinol (2·0 g.; 2 mol.) was dissolved in acetone (200 c.c.), methyl iodide (0·23 c.c.; 1 mol.) added and stirred at room temperature (22°). A slight turbidity appeared in 20 min. and gradually increased, small heavy compact prisms ultimately separating from the solution. After stirring for 5 hr. the liquid was filtered, 1·13 g. of 4-(6-*methoxyquinolyl*)-*α*-*piperidylcarbinol hydriodide* being obtained, which crystallized from water in square tablets, m.p. 214° (decomp.). (Found on the product before or after crystallization: C, 51·1, 50·9; H, 5·8, 5·7; N, 7·4, 7·4, whence C:H:N = 16 : 22 : 2. $C_{16}H_{20}O_2N_2$, HI requires C, 48·0; H, 5·3; N, 7·0 %.) The analytical figures prove that the salt has undergone slight hydrolysis with loss of hydrogen iodide. The acetone filtrate was evaporated dry under diminished pressure and the residual pale brown solid was extracted with boiling acetone, filtered from a small amount (0·13 g.) of 4-(6-methoxyquinolyl)-*α*-*N*-methylpiperidylcarbinol methiodide and the filtrate concentrated. Stout elongated prisms (0·84 g.) of 4-(6-*methoxyquinolyl*)-*α*-N-*methylpiperidylcarbinol* were obtained, m.p. 183–184°. (Found: C, 71·0; H, 7·8; N, 9·7. $C_{17}H_{22}O_2N_2$ requires C, 71·3; H, 7·8; N, 9·8 %.) The *monohydrochloride* obtained in concentrated aqueous solution from equivalent amounts of the components crystallized in long thin prisms, m.p. 103–104°. (Found: C, 53·8; H, 7·8; N, 7·7. $C_{17}H_{22}O_2N_2$, HCl, $3H_2O$ requires C, 54·1; H, 7·8; N, 7·4 %.) This salt has a neutral reaction in aqueous solution.

4-(6-*Methoxyquinolyl*)-*α*-N-*allylpiperidylcarbinol*. The parent carbinol (4·0 g.; 2 mol.) was dissolved in acetone (300 c.c.), a solution of allyl iodide, prepared from allyl bromide (0·96 c.c.; 1·5 mol.) and sodium iodide (1·66 g.; 1·5 mol.) in acetone (100 c.c.) added and stirred at room temperature for 5 hr. The hydriodide of 4-(6-methoxyquinolyl)-*α*-piperidylcarbinol (2·35 g.), m.p. 210–211°, which had separated, was collected and the acetone filtrate evaporated dry. Addition of ethyl alcoholic hydrogen chloride (2·8 c.c. of a 30 % solution) to the residue (3·3 g.) in alcohol (40 c.c.) gave 3·1 g. of 4-(6-methoxyquinolyl)-*α*-*N*-allylpiperidylcarbinol dihydrochloride, m.p. 174–175° (decomp.). This salt was dissolved in water and the regenerated base extracted with chloroform. Evaporation of the solvent and crystallization of the residue from ether gave 4-(6-methoxyquinolyl)-*α*-*N*-allylpiperidylcarbinol (1·95 g.) in diamond-shaped leaflets, m.p. 136–137°. (Found: C, 73·0; H, 7·9; N, 9·2. $C_{19}H_{24}O_2N_2$ requires C, 73·0; H, 7·8; N, 9·0 %.) The *monohydrochloride* was obtained from a concentrated aqueous solution of equivalent proportions of the components in wedge-shaped prisms, m.p. 129°. (Found: C, 62·3; H, 7·6; N, 7·7. $C_{19}H_{24}O_2N_2$, HCl, H_2O requires C, 62·2; H, 7·4; N, 7·6 %.)

Some crude specimens of *N*-allyl base on solution in hot dilute *N*-hydrochloric acid deposited fine needles which could be readily recrystallized from water and had m.p. 83–84°. These proved to be 6-*methoxyquinoline*-4-*carbinol* (XI). (Found: C, 59·0; H, 6·6; N, 6·4; OMe, 13·3.

$C_{11}H_{11}O_2N, 2H_2O$ requires C, 58·6; H, 6·7; N, 6·2; OMe, 13·8%.) It was converted into *6-methoxyquinoline-4-carbinol methiodide* (XII), m.p. 245° (decomp.), by boiling for 4 hr. with excess of methyl iodide in methyl alcoholic solution. (Found: C, 43·1; H, 4·5; N, 4·2; I, 38·9. $C_{12}H_{14}O_2NI$ requires C, 43·5; H, 4·3; N, 4·2; I, 38·3%.)

4-(6-*Methoxyquinolyl*)-α-N-*propylpiperidylcarbinol.* 4-(6-Methoxyquinolyl)-α-*N*-allylpiperidylcarbinol (1·37 g.) was dissolved in methyl alcohol (40 c.c.), platinum oxide (0·076 g.) added and the solution shaken in an atmosphere of hydrogen until absorption was complete (13 min.). (Hydrogen absorption found, 137 c.c.; calc. 112 c.c.). The platinum was filtered off and the filtration evaporated dry. The residual pale brown syrup (1·35 g.) crystallized on dissolving in acetone, and fractionation of the crystalline product from acetone gave 4-(6-methoxyquinolyl)-α-piperidylcarbinol (0·41 g.), m.p. 161–162°, and a fraction, m.p. 135–136°, crystallizing in well-formed elongated prisms which on analysis proved to be an *additive compound* of 4-(6-*methoxyquinolyl*)-α-*piperidylcarbinol* and 4-(6-*methoxyquinolyl*)-α-N-*propylpiperidylcarbinol.* (Found: C, 71·0; H, 8·0; N, 10·0. $C_{19}H_{26}O_2N_2.C_{16}H_{20}O_2N_2$ requires C, 71·6; H, 7·9; N, 9·6%.) It was found that by partial solution of this additive compound in ether and fractionation of the ether soluble portion from the same solvent 4-(6-*methoxyquinolyl*-α-N-*propylpiperidylcarbinol* could be isolated as stout rhombic prisms, m.p. 153–154°. (Found: C, 72·6; H, 8·3; N, 8·9. $C_{19}H_{26}O_2N_2$ requires C, 72·6; H, 8·3; N, 8·9%.)

4-(6-*Methoxyquinolyl*)-α-N-*propylpiperidylcarbinol monohydrochloride.* A crude fraction (1·95 g.) from the reduction product of the *N*-allyl base was dissolved in warm *N*-hydrochloric acid (6·2 c.c.) and kept at 0°. A crystalline crust (0·7 g.) separated which on recrystallization gave 4-(6-methoxyquinolyl)-α-piperidylcarbinol hydrochloride, m.p. 220° (decomp.). The mother liquors on concentration to a very small volume gave stout prisms (0·5 g.) which on recrystallization from a small volume of water separated in wedge-shaped prisms, m.p. 131°, of 4-(6-*methoxyquinolyl*)-α-N-*propylpiperidylcarbinol monohydrochloride*. (Found: H, 7·8; N, 8·0. $C_{19}H_{26}O_2N_2$, HCl requires H, 7·8; N, 8·0%.) It was not found possible to obtain constant carbon values for this substance by micro-combustion.

4-(6-*Methoxyquinolyl*)-α-N-*crotylpiperidylcarbinol.* The parent carbinol (4·0 g.; 2 mol.) was dissolved in acetone (300 c.c.), a solution of crotyl iodide, prepared from crotyl bromide (1·11 c.c.; 1·5 mol.) and sodium iodide (1·66 g.; 1·5 mol.) in acetone (100 c.c.) added and stirred for 5 hr. The crystalline deposit (2·4 g.) of the hydriodide of the parent base was collected and the filtrate evaporated dry under reduced pressure. The residual syrup (3·9 g.) was dissolved in alcohol (25 c.c.) and 4-(6-methoxyquinolyl)-α-*N*-crotylpiperidylcarbinol dihydrochloride (3·1 g.), m.p. 189–190° (decomp.), precipitated by the addition of 30 % alcoholic hydrogen chloride solution. The base was regenerated from this dihydrochloride through chloroform and on crystallization from ether gave 4-(6-*methoxyquinolyl)-α-*N*-crotylpiperidylcarbinol*, m.p. 97–98°, in stout prisms. (Found: C, 73·9; H, 8·1; N, 8·8. $C_{20}H_{26}O_2N_2$ requires C, 73·6; H, 8·0; N, 8·6 %.) The *monohydrochloride* from equivalent proportions of the components in a very small volume of water formed stout colourless prisms, m.p. 115–116°. (Found: C, 60·2; H, 7·9; N, 7·4. $C_{20}H_{26}O_2N_2$, HCl, $2H_2O$ requires C, 60·2; H, 7·6; N, 7·0 %.)

4-(6-*Methoxyquinolyl*)-α-N-*butylpiperidylcarbinol* (VI, R = OMe). The crotyl base (2·2 g.) was dissolved in methyl alcohol (50 c.c.), platinum oxide (0·089 g.) added and shaken in an atmosphere of hydrogen until absorption ceased. (Hydrogen absorption, 214 c.c.; calc. 171 c.c.) The catalyst was collected and the filtrate was evaporated dry under reduced pressure, giving a pale greenish-yellow syrup (1·9 g.). On solution in ether this deposited 4-(6-methoxyquinolyl)-α-piperidylcarbinol (0·95 g.), m.p. 159–160°, but the butyl base proved to be very soluble and was not obtained in a condition for analysis. Instead, the crude syrupy base (0·8 g.) was dissolved in an equivalent of *N*/2-hydrochloric acid and the solution concentrated slowly to a small volume. 4-(6-*Methoxyquinolyl*-α-N-*butylpiperidylcarbinol monohydrochloride* separated in small rod-like prisms, m.p. 155–156°. (Found: C, 65·9; H, 8·2; N, 7·7. $C_{20}H_{28}O_2N_2$, HCl requires C, 65·8; H, 8·0; N, 7·7 %.)

We desire to express our thanks to Miss M. Vincent, Miss A. Bishop and Dr Parr Tate, working in the laboratory of Professor D. Keilin, F.R.S., at the Molteno Institute, Cambridge, for the biological results recorded in this paper. We also gratefully acknowledge gifts of sodamide free from nitrite made by Ciba Limited and by the Dyestuffs Group of Imperial Chemical Industries, Limited, which made this work possible.

SUMMARY

Quinicine (quinotoxine), the ketonic isomeride of quinine, is inactive in bird malaria. Reduction to dihydroquinicinol (2 diastereoisomerides) with reformation of a carbinol group does not restore activity; neither is activity found in α-*N*-methyldihydroquinicinol. Consideration of these results led the authors to the synthesis of 4-quinolyl-α-piperidylcarbinol

and 4-(6-methoxyquinolyl)-α-piperidylcarbinol and their *N*-methyl, *N*-propyl, *N*-allyl, *N*-butyl and *N*-crotyl derivatives. The only compounds obtained showing antiplasmodial activity were 4-(6-methoxyquinolyl)-α-piperidylcarbinol and its diastereoisomeride *iso*-4-(6-methoxyquinolyl)-α-piperidylcarbinol. These two substances appear to be the first simple substances built on the pattern of the cinchona alkaloid molecule to show antiplasmodial activity.

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612.744.2:537.745

Oscillographic study of electron transfer by *yellow ferment* and by cytochrome *c* during muscular contraction*

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(*Communicated by D. Keilin, F.R.S.—Received* 18 *August* 1937)

Lohmann (1936) pointed out in a recent review that ultimately the energy for the contractile process of muscles is derived in preference from the combustion of carbohydrate.

The term "combustion of carbohydrate", until recently difficult to define, can now be precisely described. According to Warburg (1935; 1936), its basic feature is the transfer of electrons (or hydrogen *atoms*) from hexosephosphate to the pyridine ring of the aerobic co-ferment triphosphopyridine nucleotide, and to the anaerobic co-ferment diphosphopyridine nucleotide (co-zymase). These nucleotides transfer the electrons to *yellow ferment* (flavoprotein), and also to cytochrome *c* (Theorell, 1936).

Urban and Peugnet concluded from these findings that *yellow ferment* and cytochrome are associated with some phase of muscular contraction, since they are found in muscle, and since they would be expected to have direct access to the energy supplied by carbohydrate. The oscillographic records of single aerobic twitches, which are presented below, support their conclusion.

A correlation between cytochromes and muscular activity was postulated by Keilin (1925), who observed with a spectroscope "...In specimens (of *Galleria*)...which began to struggle constantly...the band of cytochrome gradually appeared.... When these specimens ceased to move...the bands became very faint and hardly detectable....This fact indicates that in natural conditions cytochrome is in the oxidized form, and that during exertion, however great, cytochrome becomes only partially reduced.... Cytochrome acts as a respiratory catalyst which is functional in oxidized as well as in a partially reduced form...." Keilin also established a correlation between the cytochrome content of muscle and the ability of this muscle to perform work.

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* A preliminary report (Urban and Peugnet) of this investigation was presented before the Federation of American Societies for Experimental Biology at the Memphis Meeting (April 1937).

PRINCIPLE OF THE OSCILLOGRAPHIC METHOD

The cytochromes, and *yellow ferment*, are active by electron transfer, or oxidation reduction. They show a change in the absorption spectrum during this process. *Yellow ferment* becomes oxidized by giving off electrons and this transfer is accompanied by the appearance of an absorption band at 465 mμ. On the other hand, the reduction of cytochrome *c*, effected by the acceptance of an electron by its iron atom, results in the appearance of a strong absorption band at 549 mμ (and of others elsewhere in the spectrum).

If then a material under observation be illuminated with monochromatic light the wave-length of which corresponds to that of a specific absorption band, changes of intensity of the transmitted light may, with suitable controls, be interpreted in terms of changes of concentration of the absorbing compound.

Now illuminate a muscle with light of wave-length 465 mμ, the muscle being held between parallel glass plates, to prevent any change in thickness during contraction. The light, issuing from a slit, traverses the muscle at right angles. A photocell, connected through a condenser-coupled amplifier to a cathode-ray oscillograph, instantly records any change in the intensity of this light. Such a change (a decrease) will occur at the moment when oxidized *yellow ferment* appears. If the wave-length of the light instead of being 465 mμ is 549 mμ, then a decrease in intensity will indicate the appearance of reduced cytochrome *c*. The deflexion, controlled by the photocell, of the cathode-ray beam from its straight path, as it sweeps horizontally across the face of the tube, might be termed a chemical analysis in situ, during contraction.

von Muralt (1935) also studied the changes in light transmission of muscle during contraction, using however a slow galvanometer as a recording instrument. But because he used *white* light, his results are a record of the *sum* of all the chemical and physical changes which affect light transmission.

Description of apparatus

Figs. 1 and 2 are self-explanatory.

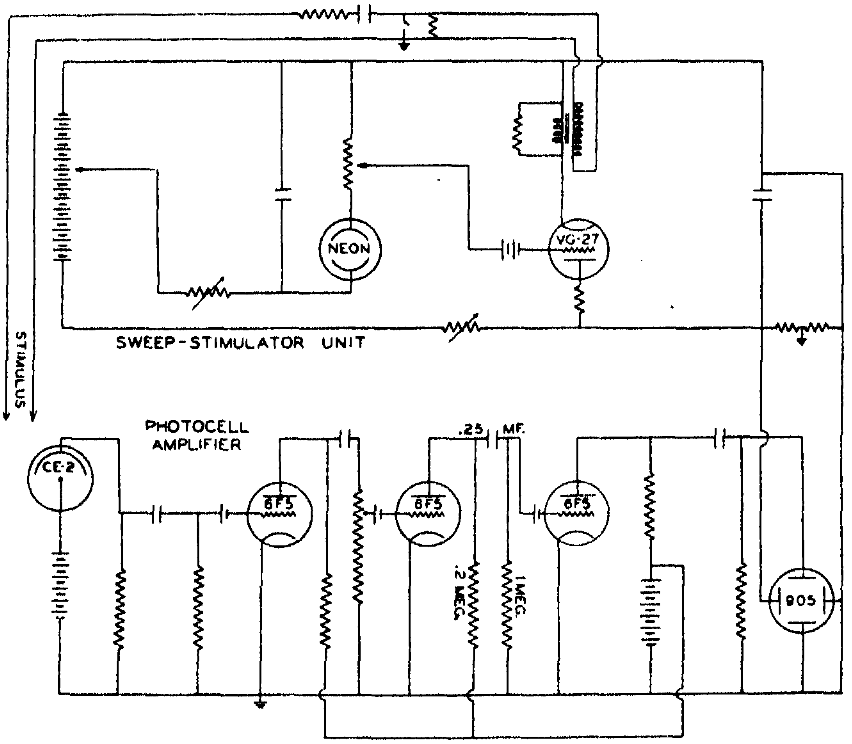

Fig. 1

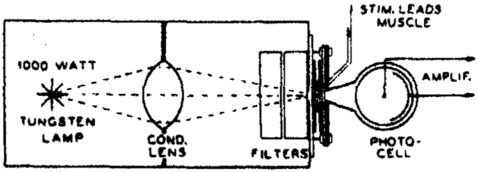

Fig. 2

It was realized that the time constants of the amplifier were too brief for exact recording of relatively *long-lasting* potentials. It must therefore be emphasized that the actual changes are of somewhat longer duration than those recorded. Except in duration this in no way affects the validity of the findings, since we are dealing with differences (see below). It is to be noted that the records shown in fig. 6 contain an angular distortion of the axes. This distortion is caused by the use of a "single-ended" amplifier output to the oscillograph. Newer apparatus includes a "push-pull" amplifier which can be made free from this distortion.

The light source (fig. 2) was a 1000 W tungsten lamp inside an air-cooled light-tight metal housing. Since the exposures were quite brief (of the order of a few seconds), air cooling was generally superfluous. Except for the experiment illustrated by fig. 5, the room temperature usually was between 20 and 22° C. The records of fig. 6 were taken in February (winter frogs).

The light from the filament was concentrated by a condenser lens, passed through filter vessels, and issued from the housing through a slit of 2 × 6 mm., as a nearly monochromatic band of light. Two parallel glass plates, the muscle lying between them, were clamped rigidly between the exit slit and the photocell. The muscle was fairly tightly held in place by lateral supports moulded of an opaque wax—rosin—lampblack mixture. With this arrangement the muscle completely filled the chamber with no reasonable possibility of thickness changes in the light path. The muscle ends were left free, in order to prevent artifacts due to longitudinal tension which were a great source of annoyance during the development of the technique. Thus it was found that longitudinal tension, whether active or passive, resulted in a decreased light transmission.

The muscle was stimulated through small wires inserted into the free ends. The glass plate adjacent to the photocell (RCA 868) was also coated with the opaque material, except for a 2 × 6 mm. slit. The photocell was completely protected from any stray light by an aluminium cover, except for a window surrounded by a hood which completely protected the exit slit from stray light.

All records were made in a dark room. The muscles were gastrocnemii of *Rana pipiens*. In order to obtain an oxygenated muscle in a "standard state" the animals were allowed to rest for 1 hr. after severance of the cervical cord and both sciatic nerves, with respiration and circulation intact. Since only the first few twitches of a freshly excised muscle, prepared in this way, were recorded, it is believed that the contractions approached *aerobic* conditions. For this reason, the findings cannot be applied to anaerobic contractions.* Nor can they necessarily be applied to summer frogs.† Two experiments with summer frogs have been unsuccessful, presumably due to the rapid oxygen depletion of the muscles at a room temperature of 30–33° C.

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* Contractions under anaerobic conditions will be discussed in a later communication. It has been our experience that results are extremely variable, unless the muscle is properly rested. Even in rested muscles, the character of the deflexion tends to change after relatively few contractions.

† Thunberg (1935) has called attention to seasonal variations in the muscular enzyme system.

The extinction coefficients of the filter solutions were determined with a Bausch-Lomb spectrophotometer.

The *yellow ferment* light filter was composed of solutions of copper sulphate, methyl violet, and reduced cytochrome *c*; these substances were kept in three separate vessels which the light beam successively traversed, before entering the muscle. The transmission curve of this filter combination, neglecting reflexion losses, is shown in fig. 3. The filter is quite satisfactory, as *yellow ferment* has a wide absorption band with a peak at 465 mμ.

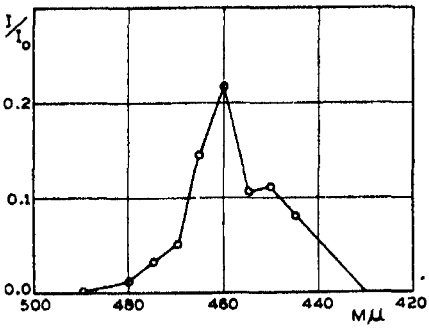

FIG. 3. Spectral transmission of the 460 mμ band (*yellow ferment* filter).

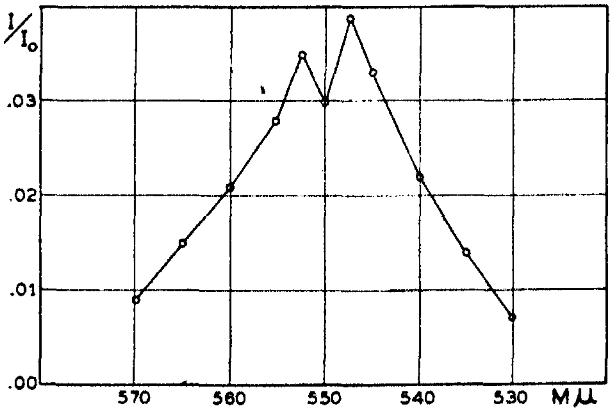

FIG. 4. Spectral transmission of the 550 mμ band (cytochrome *c* filter).

The cytochrome *c* filter consisted of solutions of copper sulphate, Lichtgruen and chrysoidin. It is apparent from the transmission curve (fig. 4) that in addition to cytochrome *c* reduced myoglobin (555 mμ) and oxymyoglobin (542 mμ) fall within the range of this filter. This is not a serious objection. Should oxymyoglobin give off oxygen during a twitch, then reduced myoglobin must appear in its stead. The absorption curves of these compounds are such (Theorell 1934) that for our filter band the oxymyoglobin-myoglobin transformation must result in a residue of *increased* light transmission; the aerobic first records taken with this light band give no indication of this (fig. 6A).

The "red control" was a fuchsine solution, absorbing completely below 625 mμ. Gray filters were used to reduce the intensity of this band as well as that of the 460 mμ band.

In order to rule out definitely any changes in the *yellow ferment* record which might be assigned to the appearance of reduced cytochrome *c* (which has an absorption band at 418 mμ in addition to the one at 549 mμ), the light band 460 mμ was transmitted through a concentrated solution of reduced cytochrome *c*, prepared from baker's yeast, before passing through the muscle.

DISCUSSION OF RESULTS

The oscillograms of muscles illuminated with *yellow ferment*, cytochrome *c* and "red control" bands are shown in figs. 5 and 6. It may be safely stated that no part of these oscillograms may be attributed to electrical "pick-up" artifacts. It was the routine practice to stimulate the muscle in the chamber in the absence of light. Under these conditions a deflexion has never been observed. (The experiment of fig. 5 was performed with newer apparatus, purposely arranged to register a stimulus artifact.)

In order to exclude the possibility that these records were due wholly to some physical factor, such as the change in light transmission produced by rearrangement of the protein structure accompanying contraction, a control oscillogram was always taken, using a broad light band extending from 625–725 mμ. This region of the spectrum was chosen as a control because no absorption bands are ordinarily seen in that region. The "non-specific" light band shows a transmission *increase** during contraction, and is thus quite analogous to the records obtained with white light by von Muralt. Since a *broad* light band in any spectral region shows the same transmission increase during contraction, it may be safe to say

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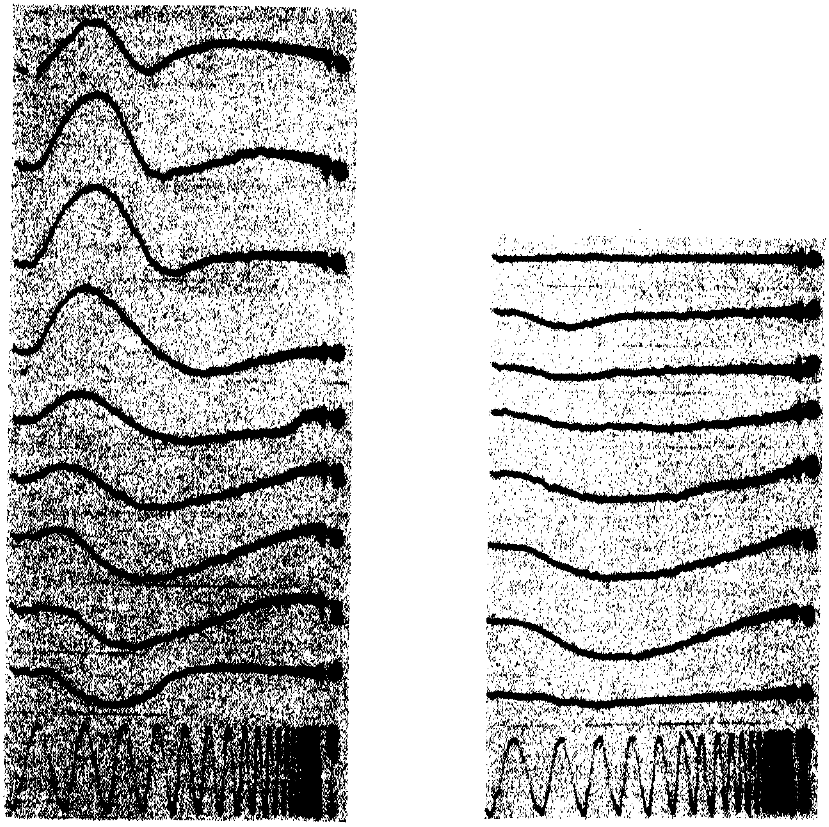
* It is worthy of note that the "red control" may instead show a decreased light transmission during contraction. Fig. 5 shows that the direction of the "red control" deflexion depends to a large extent on the state of the muscle and on the temperature. While the early contractions of a fresh muscle show an increased transmission, yet there is a progressive change to an apparently reversed picture. This reversal effect appears to be non-specific in nature, since it was also observed in other regions of the spectrum.

It is possible that this reversal effect is associated with a progressive increase in the effective tightness of the muscle thought to be due to fatigue swelling. This explanation is suggested by fig. 5B which shows a fatigue series of the companion muscle, held much more tightly in the chamber. In the latter case the deflexions are downward throughout.



A B

Fig. 5. Progressive fatigue series. Oscillographic records of changes in light transmission during contraction. R. pip. Gastroc. Direct stimulation [end to end]. Light band 625–700 $m\mu$ ["red control"]. Temp. 29° C. Timer 60 c.p.s. Note that the axes are rectangular, due to a "push-pull" amplifier.

A. Medium tight muscle chamber. Serial twitch nos. 2, 5, 7, 10, 15, 17, 20, 22, 50.
B. Companion muscle; very tight chamber. Serial twitch nos. 2, 3, 4, 5, 6, 7, 20.

that the effect is due to a physical phenomenon: possibly a decrease of light scattering during contraction. Preliminary observations of the scattered light are in agreement with this interpretation.

There is also evidence in the "red control" records of a downstroke, *following the initial rise. It is suggested that this may mark the appearance* of reduced myoglobin, as described in a recent paper by Millikan (1937). It

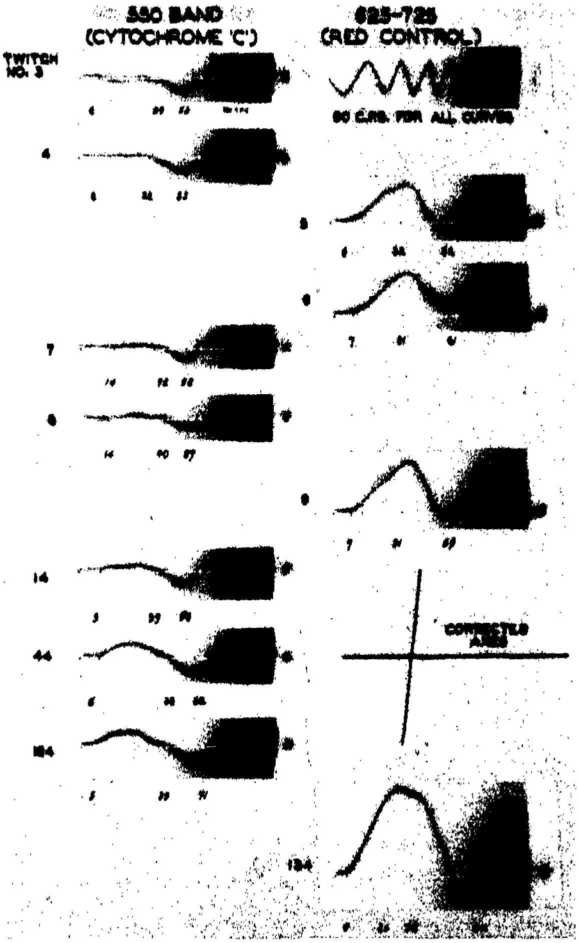


Fig. 6 A. 550 mμ band (cytochrome c) and control band. Twitch no. 13 at higher amplification.

Fig. 6 A, B. Oscillographic records of light transmission changes during contraction. Room temp. 20–22° C. Records slightly retouched. The correction of axes applies to both A and B.

is to be noted that this author employed a differential method which would not have shown the initial upstroke, assuming it to be due to a physical effect and present throughout the spectrum.

A further possibility of error is introduced by the fact that some muscles are known to act as diffraction gratings. That this error is not involved, will be clear from the facts that diffraction gratings do not destroy energy, and that the photocell measures total energy.

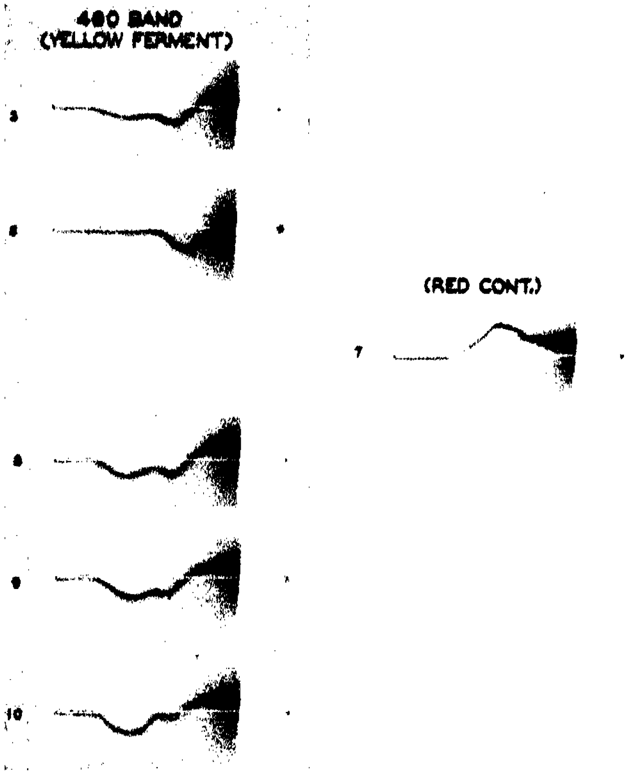


FIG. 6 B. 460 mμ band (*yellow ferment*) and control band. Different muscle.

The *yellow ferment* and cytochrome records, however, show a *decreased* light transmission during contraction. This decrease would presumably have been still more pronounced if the above non-specific effect had not existed.

The muscle is stimulated at a negligibly small interval prior to the beginning of the record. The downstroke in the cytochrome *c* pictures (fig. 6 A) is larger and earlier than in the controls. Since the earlier portion of the record is horizontal, the decreased light transmission due to the appearance of reduced cytochrome *c* must be just about compensated by the oxymyoglobin transformation and by the increase produced by the physical ("scattering") effect.

Yellow ferment (fig. 6 B) shows two downstrokes. This may be interpreted to signify that oxidized flavoprotein appears twice, indicating electron transfer at two different times in the contractile cycle. The possibility is not excluded that two different compounds are being recharged (i.e. reduced) by *yellow ferment*, and it is by no means certain that cytochrome *c* is one of the two. (The interaction of *yellow ferment*, cytochrome *c* and cytochrome *b* is at present being studied *in vitro*.)

However, inspection of the curves shows that the crest of the "red control" corresponds in time with the beginning of the second downstroke of the *yellow ferment* picture. This may mean that a continuous *yellow ferment* downward wave is interrupted by the upward wave of the non-specific "red control", producing an apparent double wave in the *yellow ferment* picture.

On the other hand, it is practically certain that the appearance of reduced cytochrome *c* during the contraction phase is *not* due to oxygen lack. The chief reason for this statement is an independent visual spectroscopic observation: a gastrocnemius showed the band of reduced cytochrome *c* after a 1–2 sec. tetanus, in the presence of a strong oxymyoglobin band. The oxygen-combining curve of myoglobin (Theorell 1934) permits us to estimate an oxygen tension in this muscle of several millimeters of mercury. But Warburg (1929) has demonstrated that the *Atmungsferment* (indophenol oxidase) is completely oxidized at 0·01 mm. Hg oxygen tension.

A second reason for excluding the possibility of oxygen lack is the fact that the third twitch clearly shows the appearance of reduced cytochrome *c*. It is difficult to see how complete oxygen lack could have developed during the very brief time which elapsed between excision and the third twitch (3–5 min.).

A third reason is based on the progressive changes shown in fig. 6 A. Here it is seen that the form of the cytochrome *c* deflexion gradually becomes grossly similar to that of the "red control", with the development of fatigue and presumably oxygen lack (excised muscles).

The objection may be raised that substances other than cytochrome *c* and *yellow ferment* may be contributing to the deflexions. This possibility may be excluded with reasonable certainty on the basis of visual spectroscopic observations.

While it is tempting to theorize on the significance of these findings for the explanation of the mechanism of muscular contraction, we shall not do so, for the present. Much remains to be done before such an interpretation can become profitable.

SUMMARY

1. An apparatus is described which records instantaneous changes in the absorption spectrum of muscle.

2. By means of this apparatus, it is shown that *yellow ferment* and cytochrome *c* are active during the aerobic contraction phase of a gastrocnemius muscle.

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612.61:597.55 *Salmo*

The male sexual stage in salmon parr (*Salmo salar* L. juv.)

BY J. H. ORTON, J. W. JONES AND G. M. KING

(*Communicated by J. Gray, F.R.S.—Received* 2 *November* 1937)

[Plate 1]

INTRODUCTION

It has long been known that ripe males occur among salmon parr, i.e. in young individuals, up to 15 cm. long, which have never left their native river. One of us (G. M. K.) has seen them frequently during the last 28 years on the Wye, Towy and Dee. These males have mostly been regarded as occasional precocious males (Calderwood 1930), and there has been no recognition that the male phase may be normal and functional in the freshwater life of salmon before they migrate to the sea. Recently knowledge of these so-called precocious males came to one of us, and in view of the peculiar biology of the salmon it was thought that a male phase might be universal and normal in salmon parr (Orton 1937) and an investigation of the problem was begun.

LITERATURE

A century ago Shaw (1840) in a paper proving that parr were the fry of salmon also noticed that male parr with ripe milt accompanied adult female spawning salmon. He concluded that the young males might be fertilizing the eggs, and showed by an experiment with milt from a young male parr and ova from an adult salmon that successful fertilizations could be made, and that the progeny became parr like the male parent. Unfertilized controls in a stream of pure water gave no fry. Further, he reared the progeny of this cross and from one young ripe male again fertilized successfully ova from an adult female salmon.

Similar experiments were successfully repeated later at Stormontfield by Brown in 1862 and by Sir Gibson Maitland at Howietown in 1880 (Day 1885). Davy in 1854 recorded the occurrence of ripe male parr. In 1885 Day summarized the information available on the breeding of salmon from parents which have never descended to the sea, stating:

"(1) that male parrs and smolts may afford milt competent to fertilize ova, but when from fish of the second season, or up to 32 months old, it is (? always) of insufficient strength for strong and vigorous fry to be raised...

(3) that young male Salmonidae are more matured for breeding purposes than are young females of the same age."

Day and his contemporary naturalists were at pains to show that salmon reared in ponds and enclosed waters could develop gonads. That discovery fostered the notion that ripe male parr are occasional precocious males. Dahl in 1910 recorded high percentages of ripe males among parr *and smolt* from the rivers of Norway and Finland, and adds that it is evident that many of these would spawn in the ensuing autumn. In 1913 Carr found that parr in the Wye, examined in May, July and October, showed male gonads varying from an undeveloped state to one almost of maturity. He states, along with McNicoll, that the ripening parr will probably remain in the river to spawn before migration. Among 252 fish he found twice as many males as females, counting individuals with a thread-like gonad (stage I) as males. Meek (1916) states that parr may take part in spawning before proceeding to sea. In 1933 Pentelow and others found two kinds of males among Tees smolts, one with very small, the other with large and well-developed testes, and that the latter migrate earlier in the season.

It is clear from this review of the literature that ripe male salmon parr (*Salmo salar*) have been found in the rivers of England, Scotland, Wales, Norway and Finland, and that many authors have expressed the view that some of the males would spawn in company with adult females in the river. Experiments have shown that such ripe male parr have functional sperm. There is uniformity in the description of all female parr stages—in the rivers—as immature. The problem remaining to be investigated is whether the male phase in salmon parr in fresh water is universal and normal before the fish proceed to the sea.

SEX-CONDITIONS OF SALMON PARR IN THE WELSH DEE IN OCTOBER 1937

By the courtesy of the Dee Fishery Board, the Superintendent and bailiffs, to whom our thanks are gladly tendered, samples of salmon parr were obtained from five localities of the River Dee and its tributaries in October 1937, at the beginning of the spawning season. The samples are random collections taken by rod and line and not selected in any other way. Length and girth measurements were made and age and sex-condition determined (see fig. 1). In recording the sex-condition the usual arbitrary phases for the development of the gonad were adopted, with the modifications and significances shown in Table I. An analysis of the gonad phases is given in Table II. Of the 226 fish examined 94 are ripe or ripening males with gonads all in the phases III to VI, 94 are young females of which 92 are in gonad phase II with ova 180 to 250μ in diameter, and 38 are of doubtful sex with long tubular gonads of phase I. These proportions are fairly maintained in the separate localities examined, except that from Hirnant brook (No. 5, Table II). This brook was nearly dry save for pools at the time of the capture of the fish and yielded no males. It would appear that the males had left the brook under the conditions prevailing; the sample is however a small one.

The fish are mainly two years old, i.e. two summers, as determined by scale-readings, and include some three years (see Table III).

As 41 % of the sample of the population of parr examined, i.e. mainly two-year-old fish, are males many of which were running and gave active sperm (see Table III), there can remain no doubt that the ripe male phase is normal in parr of *Salmo salar* at an age mainly of two years in the Cheshire Dee. The 38 fish of doubtful sex are in all probability immature males, as they have a long tubular gonad extending along almost the whole length of the abdominal cavity, whereas all the recognizable females have short gonads not reaching to the middle of this cavity. These are mainly two-year-old individuals and as some ripe males are three years old, it seems probable that a fairly large proportion of males fail to develop the gonad until the third year.

The evidence given by these samples is therefore not sufficient to permit the statement that *all* male salmon parr pass through a functional sex-phase in fresh water, but there is no doubt that the greater proportion, and possibly all, do so.

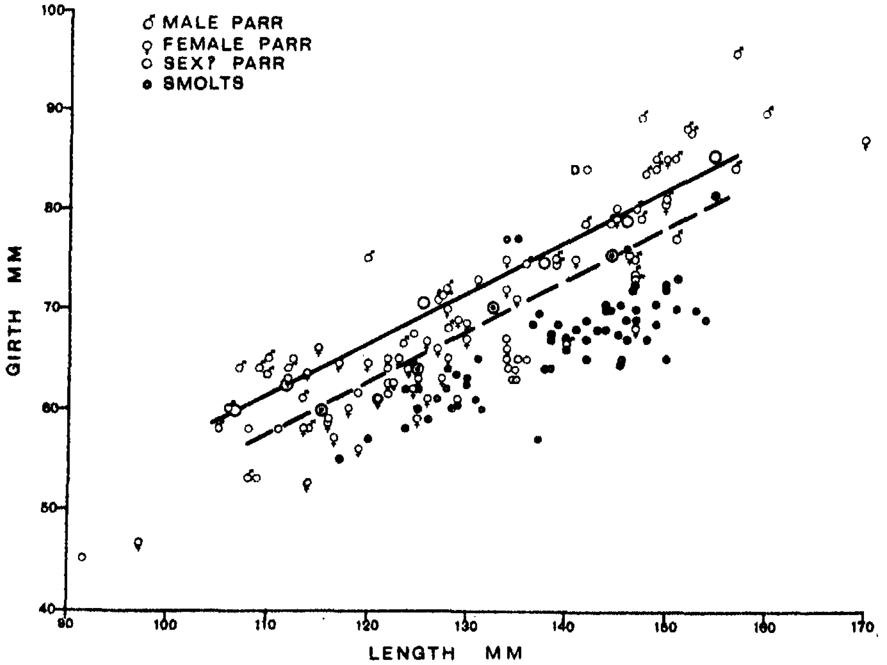

FIG. 1. Distribution of sex and size (= Length/Girth) in 106 salmon parr, *Salmo salar*, from the River Dee, Cheshire, and its tributaries, Hirnant brook, Llandrillo brook and River Ceiriog, October 1937, and size in 69 smolts from the River Dee, May 1937. (See notes on Table VI, p. 113.)

The continuous line gives the average range in size of the males, the discontinuous one that of the females. Length is measured from the tip of the snout to the fork of the tail, and girth immediately in front of the dorsal fin.

The females remain immature in the parr stage, as is reflected in their smaller girth for a given length (see fig. 1). In the sample the largest individuals are mostly males, and it may be that males grow larger than females, but a larger sample of the population is required to obtain satisfactory evidence.

COMPARISON OF SEX-CONDITION IN MATURE PARR AND SMOLTS

As the parr change into smolts which pass down the Dee seawards about May, the condition of the smolts in May will give some clue as to what has happened to the parr in the spawning season.

Fortunately samples of smolts had been collected for the study of their scales in May 1937 and a small sample was available for May 1936. These were examined for sex-condition and size. The size is plotted in fig. 1 and shown in Table V along with that of the parr; the range in length is about the same as that of the parr and the girth is shown as rather less, but as the smolts had shrunk from preservation in formalin the girth measurements are unreliable and the length is probably a little less than in the living fish. Nevertheless the smolts are about the same size as the parr, and it is reasonable to conclude that little if any growth occurs after the mature

TABLE I. ARBITRARY STAGES IN THE DEVELOPMENT OF THE GONAD IN SALMON PARR, *SALMO SALAR*

Stage 0.	No gonadial tube visible to the naked eye.
Stage I.	Gonadial tube present about 1 mm. wide.
Stage II.	Gonad about 5 mm. wide.
Stage III.	Gonad filling about half the abdominal cavity.
Stage IV.	Gonad filling rather more than half the body cavity.
Stage V.	Gonad full grown, filling the whole of the available space in the abdominal cavity.
Stage VI.	Gonad full grown and ripe gonadial elements oozing to the exterior, that is, the animal is either spawning or ready to spawn.
Stage VII.	Gonad spent.

Notes on Table I

Stage I. A definite visible small gonoduct is present in some individuals extending the length of the body cavity; in some cases this is enlarged and vascular in the anterior half.

Stage II. In the recognizable female salmon parr the gonad is developed anteriorly only with no visible oviduct in the hinder ¾ to ⅘ of the abdominal cavity. No larger stages than II have been seen. In the recognizable male in stage II the gonad extends the whole length of the body cavity.

Stage VII represents the final stage in spawning; intermediate stages of gonads diminishing from full are sometimes recognizable during the spawning season, and their grade size may be noted by that of the corresponding stage.

parr stage, even though the parr were taken in October and the smolt in May of 1937.

TABLE II. DISTRIBUTION OF SEX AND SEX-PHASES IN SAMPLES OF PARR OF *SALMO SALAR*, RIVER DEE (CHESHIRE), OCTOBER 1937

			Stages of gonad development							Totals		
Sample no.	Locality	Sex	0	I	II	III	IV	V	VI	♂	♀	☿
1	R. Ceiriog	♂	—	—	—	5+2	9+5	3+2	?	26	—	—
	53	♀	—	1	22	—	—	—	—	—	23	—
		☿	2	2	—	—	—	—	—	—	—	4
2	Llandrillo	♂	—	—	—	4+3	5+3	5+1	?	21	—	—
	brook	♀	—	—	18	—	—	—	—	—	18	—
	50	☿	—	11	—	—	—	—	—	—	—	11
3	R. Dee	♂	—	1	—	2+2	4+1	10+1	?	21	—	—
	50	♀	—	0+1	25	—	—	—	—	—	26	—
		☿	—	3	—	—	—	—	—	—	—	3
4	R. Alwyn	♂	—	—	—	2	8+2	8	6	26	—	—
	62	♀	—	—	20	—	—	—	—	—	20	—
		☿	—	16	—	—	—	—	—	—	—	16
5	Hirnant	♂	—	—	—	—	—	—	—	0	—	—
	brook	♀	—	—	7	—	—	—	—	—	7	—
	11	☿	1	3	—	—	—	—	—	—	—	4
						Totals						
		♂	—	1	—	13+7	26+11	26+4	6+?	94	—	—
	226	♀	—	1+1	92	—	—	—	—	—	94	—
		☿	3	35	—	—	—	—	—	—	—	38

Notes on Table II

(1) The sign + indicates gonads larger than the average for the group.

(2) As the samples were all examined at Liverpool, except one, the total males taken running is not known; the bailiffs reported running males in most samples.

(3) In sample 5. The high proportion of neuter individuals and absence of males from the Hirnant brook is associated with very low water, the brook being nearly dry; it seems possible that the males may have deserted the drying stream.

(4) In samples 2 and 4. The relatively high proportion of neuter individuals in these samples is interesting; it is correlated with mature males.

The sex-condition is given in Table IV; 55 out of the sample of 87 smolts were female, 16 were identified as spent males and 16 were of the type regarded as immature males, but none was found with such well-developed testes as the parr showed in October. It is possible that some of the immature gonads were of the spent male type; in the fresh condition it would probably be easier to identify these with certainty.

Although the numbers of apparently spent males are few, they afford a valuable indication that the ripe parr may actually spawn during the winter season. The high proportion of females in two of the samples suggests the possibility that the sexes may migrate separately in shoals. Sampling of shoals would give useful information.

With the help of Fishery Boards it is hoped to examine large numbers of at least post-spawning parr in the winter and smolts in the spring to obtain further information regarding changes in sex-condition during and after the spawning season.

DISCUSSION

The occurrence of a high percentage of ripe male parr at the beginning of the breeding season on the Dee leaves no doubt that the male sexual phase of parr in fresh water is normal and typical in this locality, while the occurrence of numerous ripe or ripening males on the Wye as shown by Carr, in the Norwegian and Finlandian waters by Dahl, and the prevalence of ripe males in other parts of Great Britain, virtually proves that this sex phase is normal and typical for *Salmo salar* throughout its geographical distribution. Sexual maturity is attained in the Cheshire Dee mainly at an age of two years.

Young parr are recorded by many observers as consorting in numbers up to a dozen with adult female salmon in the redd (Shaw *loc. cit.*; King 1930), and the suggestion has been made that they might be there to fertilize the ova. That this is possible can no longer be doubted. The abundance of young male parr in most rivers may be ample to fertilize the eggs of all the adult females, so that the role of the adult male salmon may not be simple. New critical observations on the relations of young male parr and large male salmon to the spawning female may disclose entirely new information regarding their behaviour. The condition of the gonad in smolts is not well known and is apparently generally considered to be undeveloped, (as shown in Table IV) but Dahl (1910) and Pentelow with his co-workers (1933) record the occurrence of males with well-developed testes in their samples. The spawning of the male parr in the rivers in which they are born cannot therefore be regarded as proved. It is hoped, however, to examine in detail large samples of smolts from the Cheshire Dee next year to determine whether the male parr spawned this season.

Early sexual maturity in males in the invertebrata is well known; it occurs in the common rock limpet, *Patella vulgata* (Orton 1928), the Gephyrean, *Bonellia viridis* (Baltzer 1914), the European oyster, *Ostrea*

TABLE III. ANALYSIS OF A SAMPLE OF SALMON PARR, *SALMO SALAR*, FROM THE RIVER ALWYN, CHESHIRE DEE, 22 OCTOBER 1937

No.	Gonad phase		Gonadial elements: Ova	Gonadial elements: Ripe sperm	Sex	Length in mm.	Age[1] in years
1	III	R.l. running[2]	—	∞ active	♂	142·5	2
2	IV	R.l.[3] running	—	,,	♂	lost	
3	IV	R.l. running	—	,,	♂	149	2
4	I		—	—	—?	125	2
5	I		—	—	—?	123	2
6	I		—	—	—?	115	3
7	IV+	R.l.	—	∞ active	♂	163	2
8	II		∞ 200μ[4]	—	♀	125	2
9	III		—	∞ not active	♂	127	2
10	V	eq. running	—	,,	♂	119	2
11	IV	R.l.	—	∞ some active	♂	150·5	3
12	V	R.l. running	—	,,	♂	147	3
13	I		—	—	—?	lost	
14	II		200μ	—	♀	115	2
15	I		—	—	—?	lost	
16	I		—	—	—?	123	2
17	VI	L.l. running	—	∞ active	♂	lost	
18	I		—	—	—?	131	2
19	V	R.l. running	—	∞ active	♂	112	2
20	I		—	—	—?	112	2
21	I		—	—	—?	114	2
22	II		∞	—	♀	130	2
23	VI	R.l. running	—	∞ active	♂	133	3
24	V	R.l. running	—	∞ some active	♂	137	3
25	VI	sub-eq. running	—	∞ active	♂	114	2
26	I		—	—	—?	107	2
27	II		∞	—	♀	113·5	2
28	V	R.l. running	—	∞ active	♂	126	2?
29	IV	eq. running	—	,,	♂	145	2?
30	II		∞	—	♀	130	3
31	VI	R.l. running	—	∞ not active	♂	150	3
32	V	R.l. running	—	,,	♂	137	3
33	II		∞	—	♀	123	2
34	II		∞	—	♀	114	2
35	II		∞	—	♀	130·5	3
36	II		∞	—	♀	120	2
37	I		—	—	—?	110	2
38	I		—	—	—?	121	2
39	I		—	—	—?	114	2
40	II		∞	—	♀	109·5	2
41	II		∞	—	♀	113	2
42	II		∞	—	♀	97·5	2
43	II		∞	—	♀	117	2
44	II		∞	—	♀	99·5	2
45	IV–V	R.l. running	—	∞ not active	♂	116	2

TABLE III (*continued*)

No.	Gonad phase	Gonadial elements		Sex	Length in mm.	Age[1] in years
		Ova	Ripe sperm			
46	I	—	—	—?	115	2
47	I	—	—	—?	100	2
48	II	∞	—	♀	107	2
49	II	∞	—	♀	123	2
50	V R.l. running	—	∞ some active	♂	128	3
51	VI L.l. running	—	∞ n.s.[5] active	♂	118	2
52	IV L.l. running	—	,,	♂	108	2
53	IV eq. running	—	,,	♂	97	2
54	II	∞	—	♀	129	2
55	VI eq. running	—	∞ n.s. active	♂	109	2
56	II	∞	—	♀	118	2
57	I	—	—	—?	116·5	2
58	II	∞	—	♀	114	2
59	V eq.	—	∞ n.o.	♂	114	2
60	II	∞	—	♀	91	2
61	IV–V R.l. running	—	∞ n.o.	♂	147·5	3?
62	V eq. running	—	,,	♂	108	2

[1] Estimated from scales.

[2] Individuals extruding milt from the sperm duct.

[3] R.l. = right gonad larger; L.l. = left gonad larger. The right is mostly the larger but is occasionally shorter than the left, and sometimes the two are about equal = eq.

[4] The ova varied in diameter from about 180 to 250μ in all the samples.

[5] n.s. = none seen; n.o. = not observed.

Note. The average number of rows of scales from the mid-dorsal line to the lateral line immediately posterior to the adipose fin is 11·3. The average number of pyloric caeca is 56·3, of which 9·4 form a separate proximal group, while that of rays in the dorsal fin is 1 spine + 12·7 branched rays, of which the last two are almost always joined at the base.

edulis (Orton 1921), the American slipper limpet, *Crepidula fornicata* (Orton 1909), and many others.

Among fishes it would seem that in current knowledge the salmon is unique in maturing as a male at a size which is insignificant in comparison to what is usually termed the adult. The phenomenon is comparable with late paedogenesis. This may occur however in other Salmonidae and notably in rainbow trout.

SUMMARY

A high proportion, about 40%, of the population of two- and three-year-old salmon parr became sexually mature in October 1937 in the River

TABLE IV. SEX-PHASES AND GONAD DEVELOPMENT IN SALMON SMOLTS FROM THE RIVER DEE, CHESHIRE

Sample no.	Date	Sex	Stages of gonad development							Totals		
			0	I	II	III	IV	V	VI	♂	♀	☿
1	15. iii. 37	♂	—	7?	—	—	—	—	—	7?	—	—
	(39)	♀	—	—	27	—	—	—	—	—	27	—
		☿	—	5	—	—	—	—	—	—	—	5
2	11. v. 37	♂	—	—	5?	—	—	—	—	5?	—	—
	(30)	♀	—	—	21	—	—	—	—	—	21	—
		☿	2	2	—	—	—	—	—	—	—	4
3	6–18. v. 36	♂	—	—	4?	—	—	—	—	4?	—	—
	(18)	♀	—	—	7	—	—	—	—	—	7	—
		☿	—	6	1	—	—	—	—	—	—	7
					Totals							
		♂	—	7	9	—	—	—	—	16?	—	—
	(87)	♀	—	—	55	—	—	—	—	—	55	—
		☿	2	13	1	—	—	—	—	—	—	16

TABLE V. SIZE DISTRIBUTION (LENGTH/GIRTH) OF SMOLTS (MAY 1937) AND OF MALE, FEMALE AND NEUTER PARR (OCTOBER 1937) OF *SALMO SALAR*, CHESHIRE DEE

	Average size (Length/Girth) in cm. length-groups									
	90–99·5		100–109·5		110–119·5		120–129·5		130–139·5	
	No.	L./G.	No.	L./G.	No.	L./G.	No.	L./G.	No.	L./G.
Male parr	—	—	5	1·78	5	1·80	6	1·78	3	1·85
Female parr	1	2·09	—	—	10	1·93	16	1·95	7	1·88
Neuter parr	1	2·03	2	1·95	4	1·88	5	1·94	2	2·03
Smolts	—	—	—	—	1	2·13	12	2·07	22	1·97

	140–149·5		150–159·5		160–169·5		170–179·5	
	No.	L./G.	No.	L./G.	No.	L./G.	No.	L./G.
Male parr	12	1·85	7	1·79	1	1·79	—	—
Female parr	5	1·91	2	1·81	—	—	1	1·95
Neuter parr	1	1·69	—	—	—	—	—	—
Smolts	26	2·11	8	2·12	—	—	—	—

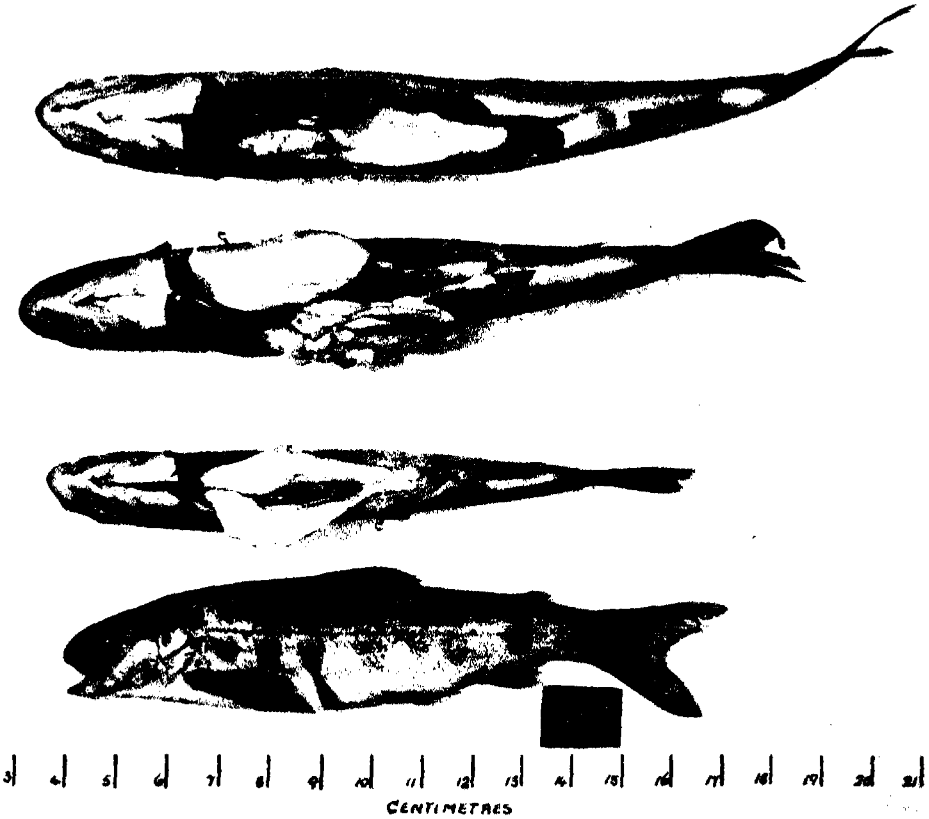

FIG. 2. Salmon parr, River Dee, October 1937, showing three males with full development of testes and ripe sperm and one female with normal parr type of ovary. In the uppermost fish the testis is shown on the right side only; in the next below the hinder parts of the testes are shown upon a background of black paper; the third one has testes of equal size; the lowest one is a female, the ovary of which is shown alongside on a black ground. Photograph by P. Bond.

TABLE VI. LENGTH AND SEX-CHARACTERS OF SMOLTS (*SALMO SALAR*) TAKEN AT CHESTER WEIR, 16 MAY 1936

No.	Length in mm.	Gonad phase	Remarks on gonad	Ova	Sperm	Sex	Remarks on sex
1	130	I	Length of body cavity	—	—	☿	Sex indeterminate
2	144	II	Less than ½ length of b.c.	∞ 200μ	—	♀	Young female
3	134	II	Length of b.c., yellow	—	n.s.	♂?	Recovering spent male?
4	114·5	II	Less than ½ length of b.c.	∞ 150μ	—	♀	Young female
5	131·0	I	Length of b.c.	—	—	☿	Sex indeterminate
6	149	II	Length of b.c., yellow	—	n.s.	♂?	Recovering spent male?
7	123	II	½ length of b.c.	∞ 150μ	—	♀	Young female
8	146	II	Less than ½ length of b.c.	∞ 180μ	—	♀	„
9	137	II	Length of b.c., white	—	n.s.	♂?	Recovering spent male?
10	141	I	Length of b.c.	—	—	☿	Sex indeterminate
11	143	II	Less than ½ length of b.c.	∞ 160μ	—	♀	Young female
12	137	II	Length of b.c., yellow	—	n.s.	♂?	Recovering spent male?
13	110	II	Less than ½ length of b.c.	∞ 100μ	—	♀	Young female
14	114	I	Length of b.c.	—	—	☿	Sex indeterminate
15	120	I	Length of b.c.	—	—	☿	„
16	118	II	Less than ½ length of b.c.	∞ 120μ	—	♀	Young female

Notes on Table VI

The fish were held up at the weir on their way to the sea and were too much shrunken for reliable girth measurements, and lengths are only approximately those in the living fish. The diameter of the largest eggs seen is given and is much less than that of the fresh eggs.

The female gonad is always short and differs from that of the male and the indeterminate gonad in this respect.

Dee, Wales and Cheshire. There is some indication that they may spawn on the immigrating adult female salmon redds before migrating to the sea as smolts. Mature and maturing male parr have been observed, in some cases constituting a large proportion of the population, in other rivers in Great

Britain, Norway and Finland, whence it is highly probable that most or all of the males of *Salmo salar* normally attain their first sexual maturity in the river in which they are reared before migrating to the sea. The phenomenon of early sexual maturity in the salmon is comparable with late paedogenesis.

[*Note added in Proof*, 16 *February* 1938. A number of experiments in fertilizing adult female salmon ova with milt from male parr were made in November. Some made by adding parr milt to ova *in water* were unsuccessful or only partially successful; others made in the usual way by adding excess of milt to the freshly extruded ova were highly successful, yielding a high percentage of larvae (alevins). The work of Shaw and others is thus confirmed and the maturity of parr sperm proved.]

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581.035.2

The effect on the growth of *Lemna minor* of alternating periods of light and darkness of equal length

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1. Introduction

Garner and Allard (1931) showed that plants grown in alternating light and dark periods of equal duration respond differently according to the length of the period. They found that the growth of *Cosmos sulphureus*, for example, was progressively poorer as the alternating periods decreased from 12 hr. to 1 min., after which it improved rapidly so that at 5 sec. intermittency the plants were of fairly normal appearance, i.e. like those in 12 hr. alternations. No measurements of these effects however were made. Portsmouth (1937) carried out similar experiments with cucumber plants, and has shown that the increase in total dry weight is greatest in continuous light, only slightly less in 12 hr. alternations and considerably less in 1 min. alternations. He suggests that the effects observed were related to carbohydrate deficiency occasioned by a falling net assimilation rate and partial closure of the stomata at 1 min. alternations. Gregory and Pearse (1937) have shown that short alternating light and dark periods are accompanied by a closing of the stomata in *Pelargonium*, and here again the effect was at a maximum with intermittencies of about 1 min. duration. In view of the above results it was decided to repeat and extend the growth-rate determinations with *Lemna minor*, which is simple in structure and is eminently suitable for experimental work under laboratory conditions. It had also the advantage, as its stomatal opening does not change, that should results similar to those of Garner and Allard, and Portsmouth, be obtained it would be possible to assess the value of stomatal closure at rapid intermittencies on growth rate.

2. Experimental procedure

Two different stocks of *Lemna* were employed, one, coming originally from the Chelsea Physic Garden, had been used previously in this department, while the other was a fresh stock obtained from Sutton. Each stock was established in the first instance from a single individual. In all cases colonies were grown under 12 hr. alternating light and darkness for 14 days before being placed under the lighting conditions in which their growth rate was to be measured. Apart from the light factor all environmental conditions were the same during the preliminary 14 days as when measurements were being made. The plants were grown in a culture solution made up as follows: $CaH_4(PO_4)_2.H_2O$ 0·100 g., KNO_3 0·800 g., $MgSO_4.7H_2O$ 0·25 g., $FeCl_3$ 0·002 g., distilled water 1000 ml. In some cases the water used was condensed on glass, in others on copper. The culture solution was changed only when the plants were being measured; it was not aerated but always shaken up with air immediately before being used. The solution was made up in amounts of 2 litres, but as the number of colonies to be supplied varied from time to time the several lots lasted for different periods.

The solution was placed in deep crystallizing dishes on which the plants floated inside paraffin-wax rings which served to keep them away from the sides. The dishes were supported in a thermostatically controlled water bath, the water in which was kept continually agitated by means of two electrically driven stirrers. The temperature of the bath was maintained at 23° C. Two sets of dishes could be used at the same time, the one set receiving different light treatment from the other. Each lighting unit consisted of four 100 watt lamps grouped close together, the light from one unit being screened off from plants under the other. Under each set of lamps was suspended a circular tank through which water was slowly passed, thus removing the excess heat from the lamps. Below the tanks were fixed compensating electric heaters consisting of open spirals of nickel-chrome wire arranged in a ring so as to cut off the smallest amount of light from the plants below them. The compensating heaters were used to supply an amount of heat equal to that emitted by the lamps, as measured at the position occupied by the plants; these were brought into operation when the light was off so that the colonies in the dishes were kept under constant temperature conditions whether the lamps were on or off. The light intensity at the position occupied by the plants was approximately 450 foot candles.

For long alternating periods of light and darkness one of the two sets of lamps and its compensating heater were controlled by means of two Venner Time Switches, which were adjusted so that lamps and heater were in operation alternately. The second set of lamps and heater were used for short alternating periods and were controlled by means of a synchronous electric gramophone motor. The latter (with a reduction gear as supplied) gave a speed of 80 r.p.m., and was used to operate a set of gears which made available a series of spindles revolving at different speeds. Any one of these spindles could be coupled to a mercury switch which controlled the lamps and compensating heater.

Dry-weight determinations were made by the method adopted by Ashby and Oxley (1935). Except where otherwise stated counts of frond number and measurements of dry weights were made at 2-day periods.

The procedure in detail for any one day was as follows. All the fronds in each dish were first washed under a jet of water and then 100 fronds were selected at random from the colony and replaced in the dish with fresh solution to carry on the experiment. The remaining fronds were counted and used for dry-weight determinations.

3. EXPERIMENTAL RESULTS

Sixteen separate experiments were made, in each of which frond counts and dry-weight determinations were carried out either daily or on alternate days for periods ranging from 2 to 62 days. The experiments are labelled A to P in Table I. In all the experiments with the exception of H the plants were from the Chelsea stock, those used in experiment H were from a Sutton strain. The culture solution in experiments A to D inclusive was made up with water condensed on copper; in the remaining experiments water prepared in a glass still was used. The use of copper-distilled water resulted from the first in a reduced rate of frond division and frond dry weight as compared with measurements in glass-distilled water, and in about 7 weeks caused injuries which ultimately had a lethal effect. In these four experiments therefore the observations of the first 21 days only have been used, i.e. before the injurious effect became apparent.

The results of all the experiments are summarized in Table I and presented graphically in fig. 1.

In the table the third column shows the average weight of 100 fronds during the whole experiment. The linear regression coefficients of log. frond number on time (column 5) were calculated by the method of least squares (Fisher 1928) and column 6 shows the mean values of these for each treatment. The value 0·164 in this column is the mean of the coefficients of experiments K and L only. In column 7, regression coefficients of log. frond number + log. dry weight (per 100 fronds) are given, i.e. the coefficients of log. dry weight on time. These were obtained by drawing by eye a straight line through the points in each experiment and finding its slope. Column 8

TABLE I

Experiment	Length of alternating periods	Av. wt. in g. per 100 fronds	Length of expt. days	Linear regression coefficient of log. frond number	Means of coefficients of log. frond number for each different treatment		Linear regression coefficients of log. dry weight	Means of coefficients of log. dry weight	
					Mean	Std. error %		Mean	Std. error %
A	12 hr.	0·0100	21	0·157	0·157	±0·0	—	—	—
D	,,	0·0083	21	0·157			—	—	—
C	1 min.	0·0058	21	0·138	0·131	±5·3	—	—	—
B	,,	0·0073	21	0·124			—	—	—
E	12 hr.	0·0141	62	0·218	0·216	±1·1	0·222	0·221	±0·8
F	,,	0·0160	42	0·218			0·218		
G	,,	0·0145	38	0·211			0·224		
H	Continuous light	0·0215	30	0·261	—	—	0·280	—	—
I	5 min.	0·0111	18	0·217	0·221	±2·0	0·213	0·214	±0·5
J	,,	0·0115	18	0·226			0·215		
K	1 min.	0·0078	28	0·162	0·164	±1·2	0·138	0·139	±0·7
L	,,	0·0089	24	0·166			0·140		
M	,,	0·0127	14	0·175	—	—	0·160	—	—
N	,,	—	4	0·198			—		
O	5 sec.	0·0108	22	0·209	0·212	±1·4	0·244	0·251	±2·8
P	,,	0·0101	22	0·215			0·258		

shows the means of these values for each experiment (again experiments M and N are not included in estimating the mean for 1 min. alternations) and their standard errors.

In fig. 1 the lowest curve shows the variation of average dry weight per 100 fronds with different alternating periods of light and darkness, the middle and upper curves show the variations of the rates of increase, i.e. regression coefficients of log. frond number and log. dry weight respectively with different alternating periods. The various treatments from left to right are: *continuous light, and* 12 hr., 5 min., 1 min. and 5 sec. alternating periods.

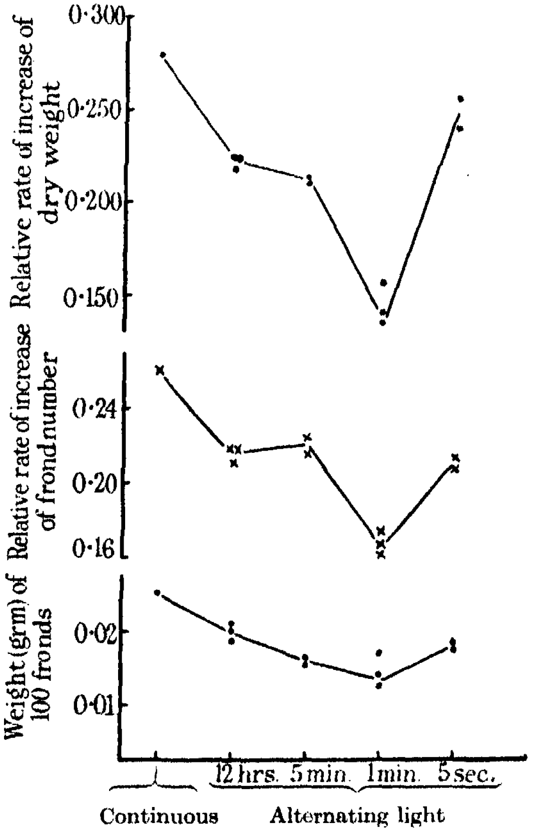

FIG. 1

A consideration of fig. 1 shows that in continuous light the average frond weight, the rate of frond division and the rate of increase of dry weight are higher than in any of the respective alternating treatments. These values, are, however, only a little greater than those in 12 hr. alternations, although the plants were illuminated for twice as long a time. The rates of increase of frond number and dry weight have respectively much the same values at 12 hr., 5 min. and 5 sec. alternations but are markedly less under 1 min. alternations. This also applies in a smaller degree to the average dry-weight curve. It will be seen then that the rate of growth, whether measured as rate of frond division, or rate of increase of dry weight, is high at 12 hr. and 5 min. alternations, falls to a minimum at 1 min. and rises again at 5 sec. alternations.

The four experiments carried out at 1 min. alternations were continued for 28, 24, 14 and 4 days, respectively, and it will be seen that as the duration of the experiment decreased the values of the average frond weight and of the coefficients of log. dry weight and log. frond number increased. The samples of plants with which these experiments (K to N) were started were taken successively from another colony—that of experiment E—growing under 12 hr. alternations. On transference to 1 min. alternations the relative rate of frond division was in all cases faster during the first few days than subsequently, and this combined with the differences in length of the four experiments accounts partly for the differences obtained. Another factor was, however, found to be present, which in this particular case caused the rate of division in experiment M to appear abnormally high. This latter factor is the presence of a long periodic variation in growth as estimated by the method here employed, which will be dealt with in subsequent papers (Dickson 1938). It is sufficient to say here that the parent colony, E, at the time of removing the sample of plants for experiment M, was in a condition such that the rate of division immediately following transference to 1 min. alternations was greater than in the case of the remaining three experiments K, L and N. It is for this reason that only the measurements from experiments K and L have been used in estimating the means, although the general effects studied are equally evident when all growth rates are used. There is no evidence that the values of the coefficients of any of the remaining experiments are subject to a variation with time comparable with that found in experiments K, L, M and N.

The effect of introducing plants to 1 min. alternating light and dark periods following culture in 12 hr. alternations is firstly to cause a rise in frond weight, which is in turn followed by a decline. This alteration in weight is accompanied by an initial relatively fast rate of division as already explained, which then decreases to a more or less constant rate. At about the time when the frond weight is at its maximum, which occurs after 8 to 10 days' exposure to minute alternations, a comparatively sudden change in the appearance of the plants takes place. Newly formed fronds have no longer the glossy surface typical of colonies in the other alternating periods, they decrease in size and in regularity of outline and do not lie horizontally but at all angles to one another. The frond weight falls to about a half and the relative rate of frond division to two-thirds of their values at 12 hr. alternations.

It is of interest to note that the relative rate of frond increase in experiment E is not constant but rises slowly throughout the 62 days during which measurements were made. The equation of the curve was found to be: $y = 2{\cdot}168 + 0{\cdot}199x + 0{\cdot}0006x^2$, where y is the log. of the total number of fronds produced and x the time in 2-day periods from the beginning of the experiment. The dry weight per frond on the other hand showed a gradual falling off, so that the log. curve of dry-weight increase was approximately a straight line. Owing to the necessary length of the experiment it has not been possible to determine whether such gradual changes are of general occurrence.

4. DISCUSSION

The growth measurements on *Lemna* at various alternating periods of light and darkness are in general agreement with the observations of Portsmouth and of Garner and Allard. McAlister (1937) in estimating the rate of carbon-dioxide assimilation in alternating light and darkness has also obtained results which were similar to these in so far as the rate of assimilation was a minimum at 1 min. and rose steadily with more rapid alternations.

Portsmouth, together with Gregory and Pearse, have shown that stomatal opening was at a minimum at 1 min. alternations, and this has led the former to conclude that at least part of the drop in net assimilation rate at this intermittency may have been due to stomatal closure. It has been found, however, that this cannot be the cause of the slower growth rate in the present instance, in so far as the stomata of *Lemna minor* are apparently inoperative. (Measurements of plants taken from continuous light and from the dark revealed a small difference in stomatal aperture, which however was not significant.) It is suggested therefore that the closure of stomata may not be effective in the lowering of the net assimilation rate under these conditions, and that both stomatal closure and a fall in net assimilation rate are the results of other changes induced by the light regime. This view is supported by observations made on *Marchantia polymorpha* (grown under conditions identical with those adopted for

Lemna in the present experiments), whose air pores do not function but remain permanently open. Under 12 hr. alternations plants of *Marchantia* grew rapidly and remained healthy in appearance up to the end of the experiment. In 1 min. intermittencies, however, rate of dry-weight increase was slower even at first than in 12 hr. alternations and soon decreased as the plants became unhealthy and ultimately died.

The effect of growing plants in 1 min. alternating periods as compared with 12 hr. alternations is, in general, to produce a slower rate of increase in dry weight. This is due to a slower rate of frond division, which is accompanied by a decreased rate of growth of individual fronds, so that while each frond is older when it produces its first daughter frond it is also smaller in area and weight than when grown in 12 hr. alternating periods.

The author is grateful to Professor V. H. Blackman for suggesting these experiments and for his advice and the numerous suggestions he has made while the work was in progress. He has also pleasure in thanking Mr W. Shaw, who constructed the switching mechanism and gear box.

SUMMARY

Apparatus has been designed to enable *Lemna* to be grown in controlled conditions and under various equal alternating periods of light and darkness ranging from alternations of 5 sec. to 12 hr.

Sixteen experiments are recorded in which the rate of frond increase and the dry weight per frond were determined continuously under several different lighting conditions for periods of up to 9 weeks' duration.

It has been shown that the relative rate of increase of dry weight is at a maximum in continuous light. Under 12 hr. and 5 min. alternating periods the rate was lower, but as the light intensity was the same in all cases, and the plants in continuous light consequently received twice the amount of those in the alternating periods, the efficiency was much higher in alternating light than under continuous illumination. The rate was lowest at 1 min. alternations but with 5 sec. alternating periods it was much higher, being even significantly higher than with 12 hr. illumination.

Curves of the relative rate of increase in frond number and of the average weight per frond were similar to those for rate of dry-weight increase, except that the rate in 5 sec. alternating periods was not significantly different from that in 12 hr. alternations.

Portsmouth (1937) attributed the small growth rate of cucumber plants in 1 min. alternating periods as probably due in part at least to closure

of the stomata. Observation shows that the stomata of *Lemna* are non-functional, so that in view of the fact that decrease in growth rate at 1 min. alternating periods is as marked in *Lemna* as it is in cucumber plants, it appears more likely that stomatal closure is a secondary effect rather than the cause of the drop in growth rate.

The appearance of the plants in 1 min. alternating periods is very different from that under other conditions of illumination. The fronds are small in size and not glossy, and lie at all angles to one another. This change in morphology takes place comparatively suddenly.

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An analysis of the "pleiotropic" effects of a new lethal mutation in the rat (*Mus norvegicus*)

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[Plates 2–5]

INTRODUCTION

The number of observable "characters" in an organism is infinite. The number of genes which control development is limited. It follows that many, perhaps most, genes must not affect only one organ or character, but several at a time. Their effect is manifold, and the term "pleiotropism" has been coined to cover this diversity of actions of a single gene. The question arises: How does a gene produce its "pleiotropic" effects?

An agent X which produces the effects a and b can do so in three different ways. X may produce a and b directly, but in two independent and different ways $\left(X \genfrac{}{}{0pt}{}{\nearrow a}{\searrow b}\right)$. Or X may produce a and b directly, but in essentially the same way $\left(X\!-\!\!\genfrac{}{}{0pt}{}{\nearrow a}{\searrow b}\right)$. Or X may produce one of its effects (a) directly, and this primary product in turn conditions the other effect (b). This is a causal chain, ($X \rightarrow a \rightarrow b$ or $X \rightarrow b \rightarrow a$), one effect being subordinated to the other and following it in time. The primary effect may still be noticeable when the secondary effect has developed; then we observe "pleiotropism" in space, whereas otherwise we observe it only in time. If more than two effects are conditioned by X, combinations of these three fundamental ways of causation may occur.

As this is a complete enumeration the scheme must comprise all kinds of "pleiotropism" whatever their ultimate cause may be, whether due to bacteria, or genes, or experimental treatment, or any other agent. It will therefore have to be investigated in which of these ways "pleiotropic" gene action is brought about. A suitable material for such an investigation is afforded by pathological mutations in higher animals, because here the wealth of information about human pathology will often be an invaluable guide as to the causal relationships of the phenomena. By inference, the conclusions reached will apply to gene action in general.

In this paper a new pathological mutation in the rat (*Mus norvegicus*) will be analysed from this point of view.

GENETICS OF THE LETHAL FACTOR

The mutant to be described in this paper I owe to the generosity of Dr Marthe Vogt (Kaiser Wilhelm Institut für Hirnforschung, Berlin-Buch), in whose rat colony it was first observed. One of two males kindly handed over to me proved to be of the constitution **aappLlSs**[*] and was therefore phenotypically a pink-eyed yellow self. When out-crossed to an unrelated black hooded female (constitution **aaPPLLss**) it produced ten self and four hooded black offspring. Three F_2 matings proved by their progeny that both parents were heterozygous for the lethal factor; all the animals, being derived from a pink-eyed parent, were of course heterozygous for **p**. The offspring produced by these pairs is given in Table I. The segregation for self versus hooded has been omitted as it is irrelevant.

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[*] The factor symbols are:

A = agouti   **a** = non-agouti
P = black-eyed   **p** = pink-eyed yellow
L = normal   **l** = lethal
S = self   **s** = piebald (hooded)

The single factor ratios for black-eyed versus pink-eyed yellow and for normal versus lethal are very close to the expectation of a 3 : 1 ratio, being 111 : 34 and 112 : 33 respectively. If these two factor pairs segregated independently of one another, one would expect a 9 : 3 : 3 : 1 ratio. The data obtained are incompatible with this assumption, there being a significant excess of the original factor combinations (black-eyed normal and pink-eyed lethal) and correspondingly a deficiency of recombinations (black-eyed lethal and pink-eyed normal). The two factor pairs are therefore carried in the same pair of homologous chromosomes; that is, they are linked. The percentage of recombinations is about 21·6, this being an average value for the two sexes.

TABLE I. **PL/pl × PL/pl**

	Black-eyed		Pink-eyed		
Mating	Normal	Lethal	Normal	Lethal	Total
A	30	6	5	7	48
C	43	6	8	9	66
E	25	1	1	4	31
Total	98	13	14	20	145
9 : 3 : 3 : 1	81·56	27·19	27·19	9·06	145

Since the single factor ratio of normal versus lethal is a close approximation to a 3 : 1 ratio, there is no indication in these data for an increased pre-natal mortality of the lethals.

[*Note added in Proof*, 28 *January* 1938. Since the above was written, more material has been accumulated. 14 matings with at least 20 young each have produced 469 normals and 110 lethals (18·99 ± 1·80 per cent). There is, therefore, a significant deficiency of lethals which is obviously due to selective elimination prior to the age at which the animals can be classified. Whether this elimination happens before or after birth remains to be investigated.]

LIFE HISTORY OF THE LETHALS

Birth weights. The young of three litters were marked individually soon after birth and weighed at 24 hr. intervals. Table II shows the birth weights in grams. The table shows clearly that there is no significant difference in the birth weights between the lethals and their normal litter mates.

TABLE II

Litter	Age	Normals	Lethals	Average normals	Average lethals
Cb	Less than 2 hr.	5·3 4·8 4·8 4·7 4·7 4·6 4·6 4·5 4·2	5·3 5·1 4·7	4·7	5·0
Ab	Less than 16 hr.	6·3 5·9 5·9 5·9 5·6	6·4 5·7 5·7	5·9	5·9
Eb	Less than 24 hr.	6·0 5·9 5·9 5·6 5·5 5·5 5·5 5·5 5·5 5·5 5·4	5·3	5·6	5·3

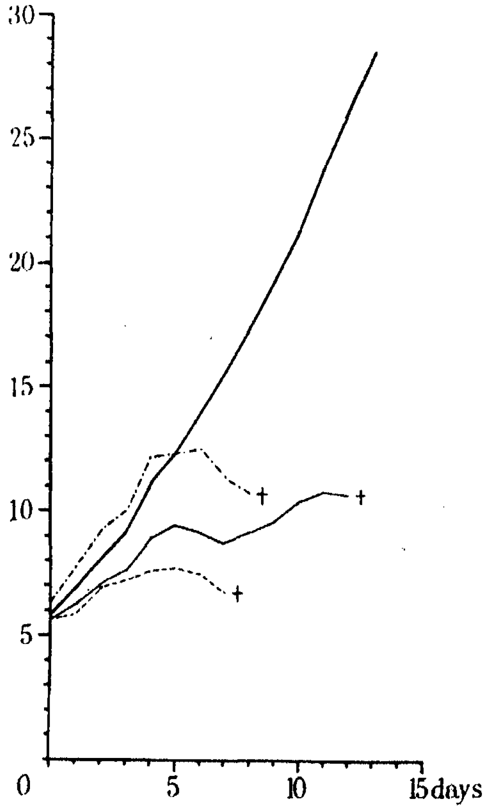

FIG. 1. Growth curves of litter *Ab*. The thick solid line is the compound growth curve of the five normal individuals.

General development. The general development and the length of life of the lethals vary widely. This is illustrated by the growth curves, figs. 1 and 2, which give the further development of litters *Ab* and *Cb* . The six lethals of figs. 1 and 2 died when respectively 7, 8, 12, 17, 18 and 29 days old. One other animal has lived up to 33 days, and 39 days has once been

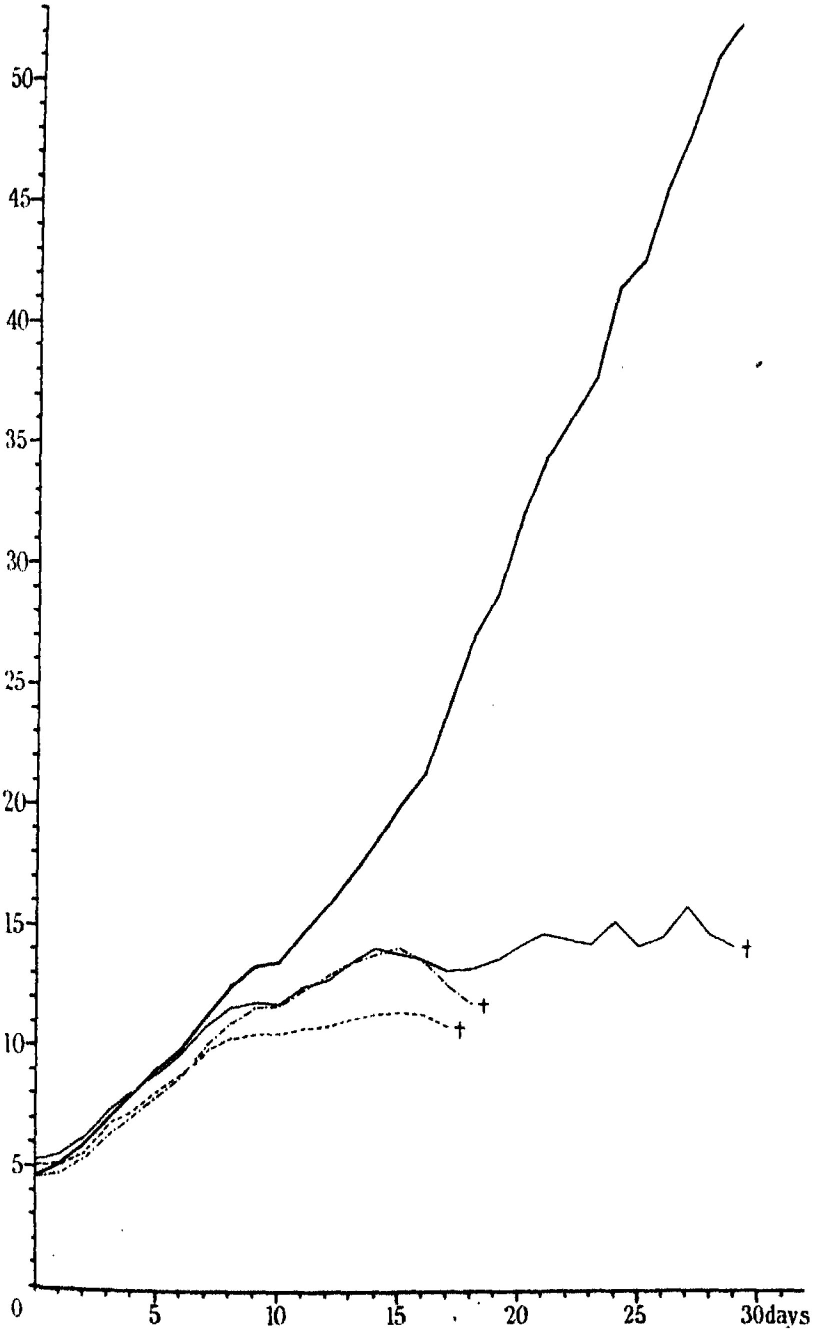

FIG. 2. Growth curves of litter *Cb*. The thick solid line is the compound growth curve of the nine normal individuals.

observed by Dr Vogt. On the other hand, several specimens have died already during the first week of life.

This excessive range of variation at once suggests that the retarding effect of our gene on growth and its eventual lethality are in fact rather remote consequences of its original action, and that several phenomena may interact which result in this variable behaviour. As will be shown below, it has been possible to elucidate the reasons for this variability in some detail.

Clinical symptoms. The snout of the lethals is very short and blunt, and the head as a whole appears to be too big in proportion to the rest of the body in subterminal stages; this is evidently a sign that the animals are losing weight towards the end. When the incisors have been cut, it is usually found that the occlusion of these teeth is faulty (see p. 135).

Older lethals are often markedly hunch-backed. There exists a rigid kyphosis of the thoracic vertebrae with a compensating lordosis of the neck and lumbar vertebrae (fig. 18, Plate 4). These strong curvatures represent an exaggeration of the normal gentle ~ shape of the spine and cannot be flattened out under anaesthesia or in a dead specimen.

The most characteristic feature of the lethals is a pronounced deformity of the chest. The thorax is strongly compressed from side to side, and at the same time its dorso-ventral diameter is greatly increased. Instead of being uniformly round and almost circular in a transverse section, it projects strongly like the keel of a boat or the breast-bone of a bird, though it is flat in the middle; the transverse section is more or less rectangular. Abnormally thick ribs are regularly visible through the thin skin. The edge of the costal arch is irregular in shape and forms a palpable, though usually shallow crest, the thorax near its lower aperture being often more or less bell-shaped.

The chest as a whole is quite rigid. A normal young rat's chest can easily be compressed laterally or dorso-ventrally between the finger-tips; but a lethal's chest is so rigid that even by using considerable force its shape cannot be altered. It cannot indeed be altered by the action of the intercostal muscles, and as a consequence the lethal rat has to do all its breathing abdominally. The abdominal breathing is, in older specimens at any rate, very violent and vigorous indeed. The beast is obviously very dyspnoeic and experiencing severe respiratory trouble. Marked cyanosis is commonly found in advanced stages.

Often, but not always, the lethals can be distinguished from their normal sibs by a peculiar noise they produce. This noise can be heard at some distance and coincides with the expiration. It seems to originate within the chest, but the exact way in which it is produced has not yet been discovered; it sounds something like ttp...ttp...ttp and has an abrupt, sharp and almost metallic character. After a varying number of these sounds (10–30 or more) a very forceful expiration is sometimes accompanied by a sound like pffshsh..., which is somewhat drawn out and cannot be perceived at a distance. These acoustic phenomena never occur in a normal young rat. Their intensity varies greatly from animal to animal and also with the age of the animal. They are usually not noticeable within the first few days of life, and tend to increase in intensity till about 10 or 12 days and may later almost disappear, if the lethal is long-lived. Generally it seems that the noisier a lethal is, the shorter is its expectation of life.

Another feature sometimes observed in young lethals is mouth-breathing. This interferes with suckling, and the shortest-lived lethals (death between 4 and 8 days) are usually such animals.

Death in some lethals takes place quite suddenly and unexpectedly; the animal becomes suddenly prostrate and violently dyspnoeic, and dies within a few hours. Sometimes the finger-nails, owing to blood congestion on the periphery of the systemic circulation, become dark red or purple. Other lethals die more slowly, and death is preceded by a period during which the animals lose weight and become increasingly comatose.

The symptoms show clearly that the reasons for death are to be looked for in the respiratory system and the circulation.

POST-MORTEM EXAMINATIONS

The most striking anomaly regularly found is an enormous thickening of the rib cartilages; in a less pronounced form, it is already noticeable at about 2 or 3 days. In a normal young rat (fig. 3) the intercostal spaces are at least twice as wide as the ribs themselves. In the lethals (fig. 3) the ribs are so thick as to be in actual contact in places, and the intercostal muscles are folded and squeezed as space permits (figs. 14 and 15, Plate 3). The thickening concerns mainly the cartilaginous part of the ribs. The bony ribs are of normal width; close to the junction with the cartilaginous rib they widen like a funnel, and at this junction the first four or five ribs actually touch one another, the intercostal space being here reduced to nil. The crowding is such that sometimes one of the first few ribs does not find a place in the row, but is partly squeezed into the thorax. The first and

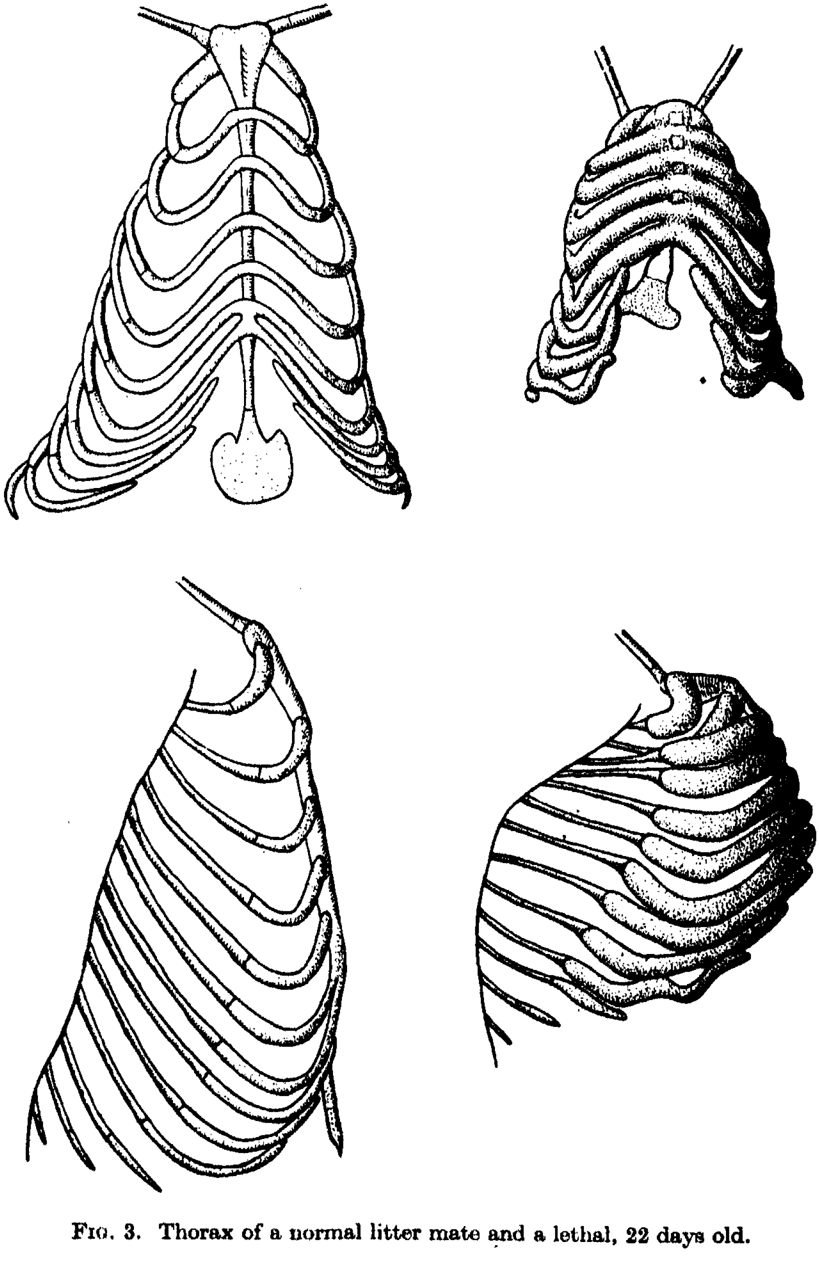

FIG. 3. Thorax of a normal litter mate and a lethal, 22 days old.

second sternebra form a distinct angle in the lethals, whereas they are practically in the same plane in normal animals.

The cartilaginous ribs are so thick that they can find a place in the row only by standing at right angles to the vertebral column. The thorax is thereby forced into extreme inspiration, no respiratory rib movements being possible. The rotation of the ribs makes the sternum project strongly in ventral direction; the dorso-ventral diameter of the thorax is thereby greatly increased, but at the same time the thoracic basket becomes compressed from side to side. Abnormal bends or kinks are often found in the region of ribs VII–X, but rarely elsewhere.

Evidently the whole thoracic basket is under stress, and the sternum will exert a counter-pressure. This counter-pressure slowly forces the thoracic vertebrae into a kyphosis which eventually becomes completely fixed and rigid.

The thoracic basket of a normal rat is a delicate mobile frame with plenty of intercostal space for the respiratory muscles. The thoracic basket of the lethals is a completely rigid carapace fixed in extreme inspiration, and its intercostal muscles are doomed to inactivity. The kyphosis appears to be a consequence of the internal stress in this abnormal thorax.

Upon opening the thorax, the lungs are hardly visible from the ventral aspect. They are largely forced behind the heart, whereas in a normal rat the lungs envelop the heart laterally and partly ventrally. A very pronounced emphysema of the lungs is regularly found. The entire lungs consist of vesicles which are often large enough to be pierced individually with a needle under the dissecting microscope. The emphysema always affects the whole of the lungs quite uniformly. Sections (figs. 7 and 8, Plate 2) show that most of the alveoli have disappeared, and that the large vesicles are the result of the fusion of a large number of neighbouring alveoli, whose vestiges are sometimes still visible as small projections from the walls of the vesicles. In well advanced cases (fig. 9, Plate 2) all the bronchi, including the intrapulmonary part of the main bronchus, are extremely dilated. The emphysema is thus accompanied by a marked cylindrical bronchiectasis; but there are never any irregular bronchiectatic sacs. The bronchiectasis accompanies only very pronounced cases of emphysema; but is lacking in slight cases, as in fig. 8, Plate 2.

Inflation of the lungs would lead to an increased volume, but the rigid chest cannot yield. The dilated parts therefore exert pressure on one another. As a consequence, some parts of the lungs are often found collapsed and entirely free of air; this happens particularly in the neighbourhood of the main bronchus (fig. 9, Plate 2) and near the edges. However, these collapsed areas are usually small.

It is obvious that both emphysema and collapse lead to a drastic reduction of the respiratory surface. No exact figures are available, but it is estimated that in the worst cases seen the surface available for respiration is reduced to about one-fifth the normal surface or less. Such a reduction of respiratory surface will slow down the development of a young organism considerably and, if far enough advanced, will completely arrest it.

The emphysematous lung is poor in blood, as the extreme rarefication of the lung tissue will lead to the closing and eventual obliteration of very large numbers of capillaries. The right side of the heart is working against an increased resistance, and an increased blood pressure in the pulmonary circulation has to be maintained to overcome this resistance.

In post-mortem examinations the condition of the heart varies to some extent, and these variations seem to be one of the factors responsible for the duration of the lethals' lives. The right auricle is regularly markedly dilated and in severe cases reaches colossal dimensions, indicating the beginning of the failure of the right ventricle. The right ventricle is often found dilated. Muscular hypertrophy occurs in varying extent. Fig. 12, Plate 3, shows a section through the heart ventricles of a lethal of 14 days of age which was already in rather poor condition when killed. The right ventricle is dilated, and there is a moderate muscular hypertrophy.

As a consequence of the increased blood pressure in the pulmonary circulation, extensive capillary haemorrhages into the emphysematous lungs occur frequently. These haemorrhages have usually sharp limits; they affect one, two, or more lobes of the lung usually completely, whereas the rest is unaffected. The affected parts are filled with blood and practically entirely free of air. Fig. 10, Plate 2, shows such a left lung taken from a 14-day-old lethal which suddenly became violently dyspnoeic and comatose and was killed when obviously *in extremis*. In this particular animal the whole of the left lung was put out of action, while the right lung was not affected. In such cases loss of blood and further restriction of the respiratory surface soon prove fatal.

In other lethals which have died spontaneously, no haemorrhages in the lungs are found. Here extreme dilation of the right side of the heart and often congestion of blood under the finger-nails are the dominating features. In these cases death is evidently due to failure of the heart to cope with the resistance in the pulmonary circulation. The failure of a heart with a compensatory muscular hypertrophy seems usually to take place fairly suddenly within a few hours, and venous congestion rapidly spreads beyond the dilated right atrium and is eventually seen at the very periphery of the systemic circulation under the finger-nails.

The rapid death of the lethals is therefore usually either due to haemorrhages into the lungs or to cardiac failure. In either case, the increased blood pressure in the pulmonary circulation is responsible.

Some lethals die more slowly, and neither haemorrhages in the lungs nor very marked signs of congestion are found. In such cases the chronic and increasing shortage of oxygen seems to be primarily responsible, though cardiac weakness is doubtless a contributing factor.

A very characteristic anomaly is regularly found in the trachea and the extrapulmonary parts of the main bronchi. The cartilaginous rings in the trachea of a normal young rat (fig. 16, Plate 3) are delicate and thin; they are almost elliptical, and so is the lumen of the trachea. In the lethals (fig. 17, Plate 3) there is an enormous hyperplasia of these cartilages, which assume the shape of a very swollen horse-shoe and narrow down the lumen considerably.

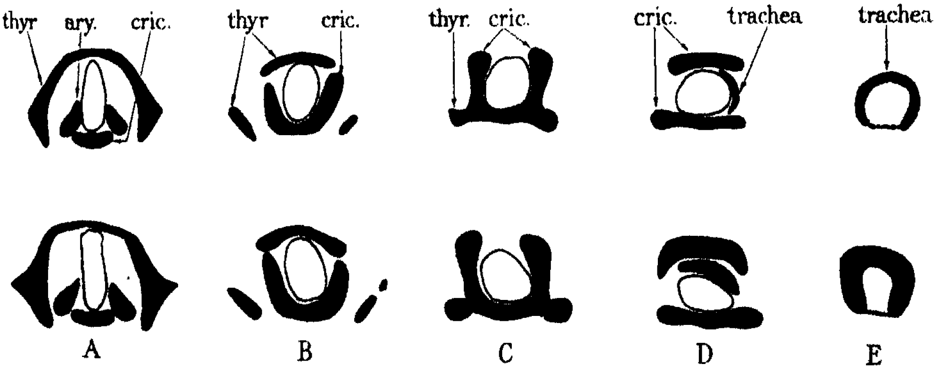

FIG. 4. Camera lucida drawings of corresponding sections through different levels of the larynx and trachea. Upper row normal sib, lower row lethal, 7 days old. Same animals as in figs. 7, 8, Plate 2. *thyr.* = cartilage thyreoidea; *cric.* = cart. cricoidea; *ary.* = cart. arytaenoidea.

In contrast to the hyperplasia of the cartilage in ribs and trachea the cartilages of the larynx (fig. 4) are very much less affected. The cricoid cartilage is slightly heavier in the lethals, but not strikingly so; similarly the thyroid cartilage is somewhat broader in the lethals, but, again, the difference is not striking.

The nasal cavities do not show any gross abnormalities. There is no noticeable hyperplasia of the nasal septum or of the cartilages of the nose.

However, the nostrils in young lethals are often more or less completely blocked by some kind of rubbish. These plugs consist mainly of plant material, derived from the hay and sawdust of the nest. The explanation is simple. A normal young rat can rid its nose of foreign bodies by sneezing. Sneezing involves a sudden explosive release of air after a deep inspiration. In the lethals, the emphysema of the lungs allows neither for a really deep inspiration nor for an effective sudden expiration. Lethals which fail in some way to rid their noses of the dirt are prevented from effective suckling and soon die of starvation.

No pathological conditions were found in any of the other inner organs. In animals with extensive haemorrhages in the lungs, some anaemia of the internal organs, particularly of the liver, is found.

The fate of an individual lethal depends on the interaction of the factors outlined above, and it is therefore not surprising that the behaviour of the lethals is so very variable.

In this connexion the case of one lethal, which reached the age of 33 days in remarkably good condition, is of some interest. On that day the animal was accidentally killed by anaesthesia when X-ray photographs were to be taken; its weight had been 15·2 g. at 15 days and 21·6 at 33 days (control 27·6 and 54·5 g. respectively). The post-mortem examination showed that the emphysema in this animal was comparatively slight; it was in fact slightly less pronounced than that of the 7-day-old specimen shown in fig. 8, Plate 2. As a consequence, the strain imposed on the heart was not so excessive, and accordingly a very good compensatory hypertrophy of the right side of the heart (fig. 13, Plate 3) had developed, there being no appreciable dilation. If it is added that this individual had its nostrils clear and that it had managed to bring its teeth into grinding contact by grinding the incisors down almost to the level of the gums (figs. 24 and 28, Plate 5), it is plausible that this favourable combination of circumstances allowed the animal to progress relatively more favourably than most of its kind.

The emphysema of the lungs is directly or indirectly the reason for death in the lethals. The next question is that for the origin of the emphysema. It appears that several facts are acting together to that end. The abnormal shape of the thorax and the kyphosis leads to a very abnormal situs of the thoracic viscera. Any dislocation of the lungs will, if anything, make respiration more difficult, never easier. Secondly, the fixation of the thoracic basket in inspiration will lead to a drastic reduction of the respiratory movements; this is bound to lead to dyspnoea, and dyspnoea in itself leads to emphysema. Finally, the narrowed lumen of the trachea and of the main bronchi will be a contributory cause, as respiration through a narrow tube is more difficult than through a wide one.

Most of these anomalies have been held responsible for the development of emphysema in man (Aschoff 1919). Their combination as observed in the lethal rat therefore explains fully why emphysema develops in these animals. All the anomalies of thorax and trachea are directly or indirectly conditioned by an anomaly of the cartilage. The next step will therefore be an investigation of the bony and cartilaginous skeleton of the lethals.

THE BONY SKELETON OF THE LETHALS

The bony skeleton of the lethals does not differ from that of their normal litter mates as long as the lethals are gaining weight normally. As soon as the divergence starts, the longitudinal growth of the long bones is slowed down and eventually completely arrested.

Similarly the first radiographical appearance and the growth of some of the ossification centres is delayed or completely arrested, though the centres differ very much in their behaviour. No detailed descriptions are to be given here; a number of details are visible in the X-ray photographs, figs. 18–20, Plates 4 and 5.

The influence of arrested growth on the skull is of some interest in view of secondary effects on the incisors. In normal animals, from the age of one week onwards, the skull undergoes a drastic elongation (figs. 21, 23, 25, 27, Plate 5). The rounded forehead of the baby rat becomes flat and the snout long and pointed. At 5 days (fig. 21) the intermaxillary bones project only slightly over the symphysis of the mandible. Rapid heterogonic growth changes the outline of the mouth cavity as seen in X-ray photographs so that the point of eruption of the upper incisors is far in front of that of the lower ones. When the incisors erupt, they are therefore in the right relative position for gnawing; the cutting edge of the lower incisors grinds against the upper incisors from below and behind.

In the lethals (figs. 22, 24, 26, 28, Plate 5) the skull proportions are entirely normal up to the age at which the divergence of development starts. Now bone growth becomes arrested or greatly slowed down, the elongation of the snout does not take place, and the skull retains its baby-like proportions. But the eruption of the teeth is not affected. As a consequence, the tips of the lower incisors just face those of the upper incisors instead of being behind them. If at this stage the lethal at once starts grinding down its teeth and keeps them always very short near gum level, the teeth can maintain grinding contact and the animal can feed. This happened, for instance, in the lethal, figs. 24 and 28, Plate 5. Usually the tips of the lower incisors come to lie in front of the upper incisors, and once this has happened the teeth cannot be worn off by use (fig. 26, Plate 5). Then the incisors continue elongating till the animal dies; since the feeding trouble in itself weakens the animal and hastens the end, the dimensions reached by the teeth are never very spectacular.

Histologically, the bone appears to be quite normal. In the epiphysial disk no mitotic figures were found in the zone of proliferation of a 14-day-old lethal, whereas numerous mitotic cell divisions in these cartilage cells were present in corresponding preparations of a normal litter mate. The zone of flattened cells arranged in columns is thinner in lethals than in normals. The cells themselves are extremely flat and shrunken, the nuclei pyknotic. The histological situation is very similar to that which Harris (1933, figs. 28–30) obtained in starving puppies. In fact, it is exactly what one would expect in a case of arrested growth.

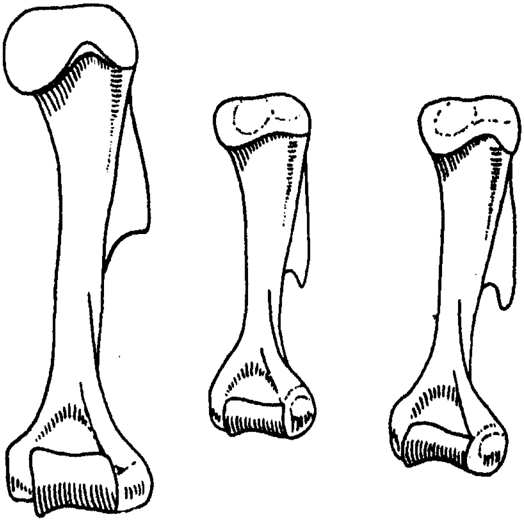

FIG. 5. Humerus of normal sib, 30 days old (left), lethal, 30 days old (middle) and lethal, 33 days old (right).

One feature of the skeleton needs special mention since, unlike everything else, it cannot be explained in terms of retarded or arrested growth. This feature is shown in fig. 5 and is also visible in the X-ray photographs, figs. 19 and 20, Plate 5. In the lethals the deltoid ridge of the humerus has a very characteristic hook or spur at its distal end. Such a spur does not occur in normal rats at any stage of development; it is not yet noticeable in X-ray photographs of very young animals, but has been constantly found in older lethals. The free edge of the ridge is thereby made longer and at the same time it seems to be somewhat thicker and stronger than in normal animals.

The reason for the existence of the spur is somewhat obscure. It may be suggested tentatively that it is in some way connected with the abnormal thoracic shape of the lethals. The free edge of the deltoid ridge serves for the insertion of the musculus pectoralis major. It may be that in the lethals that muscle owing to the altered shape of the thorax is working under different conditions and that it therefore requires a more extensive space for its insertion on the humerus.

THE CARTILAGE ANOMALY OF THE LETHALS

A preliminary histological study of the cartilages of ribs, larynx, trachea and nose shows certain abnormal features that are common to all of them, though as regards others they differ. This makes it virtually certain that at least these common features are co-ordinated, that is to say, they are the common expression of a uniform gene action produced by the same mechanism in different parts of the body.

The nature of this fundamental disturbance is beyond the scope of this paper, as it stands at the root of the "pleiotropic" system with which we are here concerned. A detailed study of cartilage histology and its behaviour *in vitro* in the lethals is planned in collaboration with the Strangeways Research Laboratory, Cambridge, and will be communicated later. A few remarks may be made here about the ribs, where the histological situation is very characteristic. The perichondrium in places is turned into a cell-rich tissue with few collagen fibres. A very intense but irregular cell proliferation takes place here; flat knobs or lenses of cartilage project over the surface of the rib; some such projections are already visible in the low-power photograph, fig. 15, Plate 3. The cartilage formed is very rich in cells, but poor in matrix; numerous binucleate cells occur in it, which are not found in normal cartilage. Towards the centre of the rib, large numbers of cartilage cells degenerate, and extensive cell-free areas are formed. The situation is similar in the trachea, though it differs in some details.

The anomaly is already present at birth, though it is too slight to be made a basis of scoring by macroscopic methods. A freshly born litter of 12 was classified histologically (ribs) as consisting of 8 normals and 4 lethals. A confirmation for the accuracy of this classification was obtained, when Dr H. B. Fell, to whom I showed the preparations (marked by numbers only), separated the slides out into precisely the same groups of 8 and 4 as I had done. As there are $\frac{9\times 10\times 11\times 12}{1\times 2\times 3\times 4} = 495$ different ways of selecting 4 objects out of 12, there is no reasonable doubt that the cartilage anomaly is present at birth. Its actual onset in pre-natal life has yet to be determined.

So far as can be judged at present, the cartilage anomaly of our lethals does not correspond to any of the recognized pathological entities in man or the domesticated animals. In particular, it appears to be totally different from rickets and from "foetal rickets", now more appropriately called achondroplasia. The latter condition, which is known as an inherited anomaly in man (Rischbieth and Barrington 1912), cattle (Crew 1923) and chickens (Landauer 1931, 1933), is characterized histologically by a more or less complete absence of cartilage columns in the growth zone of the long bones and by mucoid degeneration of the matrix (Harris 1933). Neither of these anomalies is found in our animals. It seems that overgrowth of the rib cartilages does not belong to the classical picture of achondroplasia. There seem to occur, however, certain atypical cases in which some thickening of the ribs is found; such cases have at the same time only moderately shortened limb bones; a skeleton of this kind is illustrated by Rischbieth and Barrington (1912, Plate U, fig. 18). As there seem to be no references as regards the cartilage histology of such atypical cases it remains uncertain whether the resemblance to our rats is only superficial or indicates a closer relationship.

The lethal factor described in this paper is certainly different from the only other lethal known in the rat (Crew and Kon 1933). The mode of action of that gene is unknown. However, the growth curves in Crew and Kon's case showed a very uniform behaviour, and very characteristically there was a lack of any period of stagnation and a very short and uninterrupted decline. The pathological mechanism underlying such a curve must necessarily be very different from that in our case.

THE CAUSAL RELATIONSHIPS OF THE EFFECTS

The analysis of the pathological symptoms given above allows us to bring some order into the multitude of "pleiotropic" effects produced by our lethal factor. The diagram (fig. 6) illustrates the probable causal relationships of the different effects.

This picture may not be correct in all its details, but it certainly is correct in principle.

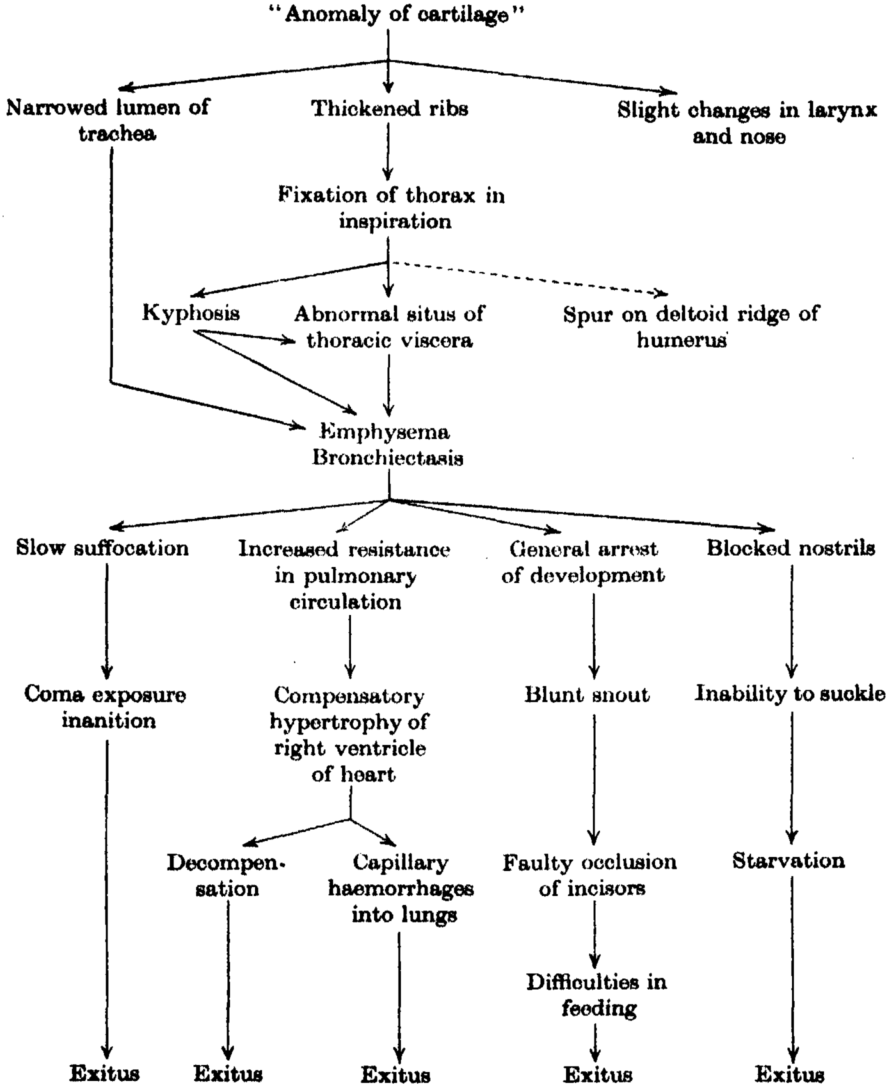
"Anomaly of cartilage"
Narrowed lumen of trachea
Thickened ribs
Slight changes in larynx and nose
Fixation of thorax in inspiration
Kyphosis
Abnormal situs of thoracic viscera
Spur on deltoid ridge of humerus
Emphysema
Bronchiectasis
Slow suffocation
Increased resistance in pulmonary circulation
General arrest of development
Blocked nostrils
Coma exposure inanition
Compensatory hypertrophy of right ventricle of heart
Blunt snout
Inability to suckle
Decompen-sation
Capillary haemorrhages into lungs
Faulty occlusion of incisors
Starvation
Difficulties in feeding
Exitus
Exitus
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FIG. 6. Diagram showing the probable causal relationships of the "pleiotropic" effects.

It will be noted that those effects which are more directly due to the basic disturbance are completely constant, being regularly found in all the homozygotes. Effects far removed from the "original" gene action tend to become more and more variable; they may develop to a greater or lesser extent or may occasionally be missing altogether. The remoter consequences are clearly more liable to be influenced by chance environmental factors than the "original" disturbance. In our case the reasons for the variability of the remoter consequences are fairly clear, as the causal chains leading up to them are known. With many genes, this is not the case. The basic disturbance is usually more or less completely concealed, and the "characters" which we subject to a Mendelian analysis are presumably often very far removed from the original gene action. Such variable characters are often referred to in the literature as due to "weak" genes with incomplete manifestation. It seems likely that a gene is never "weak" as regards its basic action, though this will often, perhaps usually, be unobservable, and that only the remote consequences of gene action are liable to modification.

When speaking of "original" gene action, it must be kept in mind that this is only the earliest stage to which the gene action has been traced back. But it is quite likely that that effect itself is only the end-product of earlier happenings concealed to morphological methods. Possibly the gulf between the true beginning of gene activity and its end-effects can never be bridged completely by morphological methods; if genes act chemically, the true primary action may always be invisible, and everything demonstrable morphologically would be only secondary.

A partial answer can now be given to the question raised in the introduction: How does a gene produce its "pleiotropic" effects? Three possibilities were suggested, namely direct action, but different methods, direct action with the same method, and lastly indirect action with subordinated effects (hierarchy of causes). It is now clear that the latter two possibilities are realized in the case under discussion. The basic action on the cartilage manifests itself in ribs, trachea, larynx, etc. These effects are clearly coordinated; the same mechanism affects several structures. The development of the emphysema of the lungs, on the other hand, is a consequence of the cartilage anomalies and therefore only indirectly conditioned by the original gene action. The emphysema again is followed by a long chain of secondary and tertiary disturbances with repeated branching off of new "side-chains".

Both possibilities have been observed previously. As an example for different gene effects produced directly in different parts of the body by the same mechanism one may quote K. Bonnevie's excellent embryological analysis of the foot and eye anomalies in Little and Bagg's mouse strain (1932, 1934). The hierarchy of causes has been admirably worked out both anatomically and physiologically in the frizzle fowl by Landauer and

Upham (1936). These two principles of "pleiotropic" gene action can therefore be regarded as safely established.

The question arises whether the third possible way of "pleiotropism", namely the direct action of one gene in entirely different ways, is ever realized. This question is of high theoretical importance. In the established types of "pleiotropism" of gene action the *unity of gene action* is maintained. There is only one process, manifested in different parts of the body, and/or chains of secondary effects. In this sense the "pleiotropism" discussed may be regarded as a *spurious* pleiotropism. A genuine pleiotropism would exist in such cases only where there was no unity of gene action, where one and the same gene directly did different things in different ways.

I am not aware of any such case, and it seems that the occurrence of genuine pleiotropism will be hard to prove. For instance, in the grey-lethal mouse (Grüneberg 1935, 1936, 1937, 1938) one single recessive gene causes absence of yellow pigment in the fur and various anomalies of skeleton, teeth, thymus, etc. With the exception of the pigment anomaly, everything can, at least tentatively, be linked up and subordinated to one basic cause, a complete lack of bone absorption; only the absence of yellow pigment does not at all fit into the picture. It would, however, be entirely wrong to regard this case as a proof for the existence of genuine pleiotropism, for it is quite possible that further back in development the apparently unconnected phenomena may converge to one single unknown process. This consideration seems to apply to all the cases, where an analysis of manifold gene effects has failed to reveal the unity of gene action. But the number of cases where "pleiotropism" has been studied in the making is very small indeed, and cases of genuine pleiotropism may yet be discovered. But as the conception of the unity of gene action is the simpler assumption, and since unity of gene action has been established in fully analysed cases, the *onus probandi* will rest with those who may deny it. But unless and until the unity of gene action has been disproved as a general principle, it seems to be both logical and in the interest of progress to regard as incompletely analysed all these cases in which the unity of gene action has not been demonstrated.

Recently, Landauer and Upham (1936) in their thorough analysis of the "pleiotropic" effects produced by the frizzle gene in chickens have come to conclusions similar to those presented in this paper, and their view that the "term pleiotropism is of little, if any, value" can only be endorsed. The term would convey a meaning only in cases of genuine pleiotropism whose existence remains still to be demonstrated.

ACKNOWLEDGEMENTS

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SUMMARY

"Pleiotropism" of genes can theoretically arise in three different ways. Two effects, *a* and *b*, may either be produced by the gene directly by means of two different mechanisms; or they may be produced by the gene by means of the same mechanism; or the gene may produce only one effect *a*, which in turn conditions *b* (indirect gene action). In the latter two cases, unity of gene action is maintained (spurious pleiotropism), whereas only the first case would constitute genuine pleiotropism, the gene producing manifold effects in different ways.

The analysis of the manifold effects of a new recessive lethal factor in the rat, which is linked to pink-eye dilution, shows that the most fundamental disturbance so far discovered is an anomaly of the cartilage; this single mechanism affects several structures of the body in a similar way. Hyperplasia of the ribs and of the cartilage rings of the trachea thus produced leads secondarily to the development of an emphysema of the lungs, and the animals ultimately die from more or less remote consequences of this emphysema.

Unity of gene action is therefore maintained in this case, which, despite the complex ramifications of the effects, is only an example of spurious pleiotropism. This seems also to apply to the few other cases which are fully analysed. The existence of genuine pleiotropism has never been conclusively proved. As unity of gene action is the simpler conception, and as it has been shown to explain fully analysed cases, it may be adopted as a general principle unless and until the occurrence of genuine pleiotropism can be conclusively demonstrated.

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EXPLANATION OF PLATES

Plate 2

FIGS. 7, 8. Left lungs of normal litter mate and lethal, 7 days old. The animals weighed 11·77 and 11·33 g. respectively; the lethal was therefore not yet appreciably back in development; X-ray photographs showed no difference in skeletal differentiation except for the thorax deformity of the lethal. Moderate emphysema in the lethal. Suza fixation. Haematoxylin-eosin. 10 μ. 8 times enlarged.

FIG. 9. Left lung of lethal, 14 days old. Very poor condition. Extreme emphysema and marked bronchiectasis; collapse of lung tissue in the neighbourhood of the enlarged main bronchus. Same technique.

FIG. 10. Left lung of lethal, 14 days old (not the same specimen as in fig. 9). This animal became suddenly prostrate and violently dyspnoeic a few hours before it was killed. Extensive fresh haemorrhages affecting almost the whole organ. Same technique.

Plate 3

FIG. 11. Transverse section through the heart ventricles; normal litter mate, 14 days old. Same technique.

FIG. 12. Transverse section through the heart ventricles, lethal, 14 days old (same individual as in fig. 9). Considerable dilation and moderate muscular hypertrophy of the right ventricle. Same technique. The differences in muscular development of the heart are rather more striking in post-mortem examinations than in sections.

FIG. 13. Transverse section through the heart ventricles, lethal, 33 days old. Long-lived and well-developed individual (same as in figs. 24, 28). Definite muscular hypertrophy of the right ventricle, no dilation. The emphysema in this animal was very moderate, being rather less pronounced than that shown in fig. 8.

FIGS. 14, 15. Longitudinal sections through the thorax wall of a normal litter mate and a lethal, 14 days old (same animals as in figs. 9, 11, 12). Suza. van Gieson. 10 μ. 8 times enlarged.

FIGS. 16, 17. Transverse sections through the trachea of the same pair of animals. 25 times enlarged.

Plate 4

Fig. 18. X-ray photographs of a lethal (left) and a normal litter mate, 23 days old. Pronounced kyphosis in the lethal. Same animals as in figs. 25, 26.

Plate 5

Figs. 19, 20. X-ray photographs of a normal litter mate (left) and a lethal. 15 days old. Same animals as in figs. 23, 24.

Figs. 21, 22. Head of normal sib and lethal, 5 days old.

Figs. 23, 24. Head of normal sib and lethal, 15 days old.

Figs. 25, 26. Head of normal sib and lethal, 23 days old.

Figs. 27, 28. Head of normal sib and lethal, 33 days old. Same animals as in figs. 23, 24; the heart of this lethal is shown in fig. 13.

595·772 *Myzus*: 576.858

Further studies on the relationship between Hyoscyamus virus 3 and the aphis *Myzus persicae* (Sulz.) with special reference to the effects of fasting

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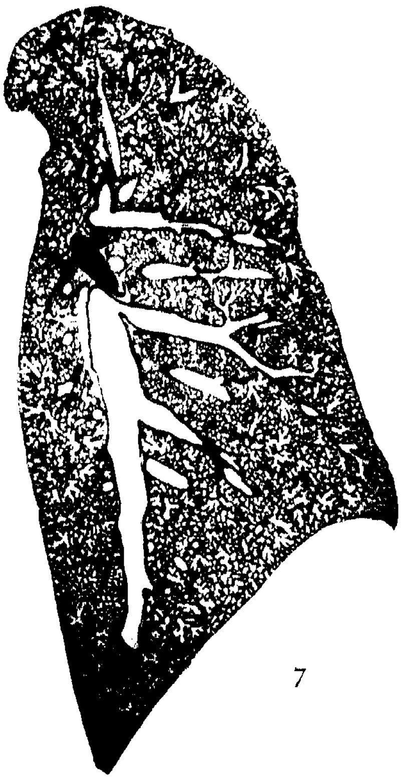
(*Communicated by Sir John Russell, F.R.S.—Received* 29 *November* 1937)

The means by which insects transmit plant viruses are largely unknown. It is improbable that the viruses are transmitted merely by contamination of the mouthparts of the insect, because there appears to be a close specific relationship between the viruses and their vectors. For this reason it has usually been assumed, on analogy with insect transmitted animal disease such as Malaria, that the virus is taken in by the vector through the mouth, passes through the wall of the alimentary canal into the haemocoele, and is introduced into a new host with the saliva. There is good evidence to suggest that at least one class of viruses is transmitted in this way, but only the viruses causing Streak disease of Maize, and Curly-top of Sugar beet, have been shown to be present within their insect vectors, and of these only the first has been obtained from the blood stream. So far no



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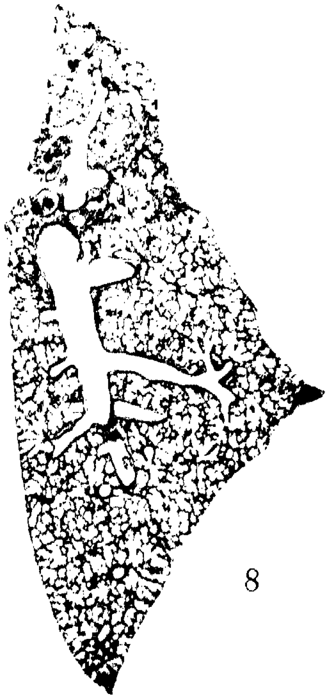

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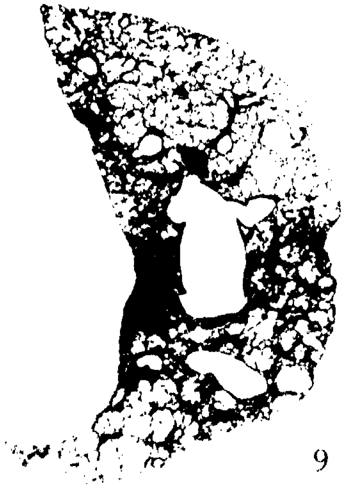

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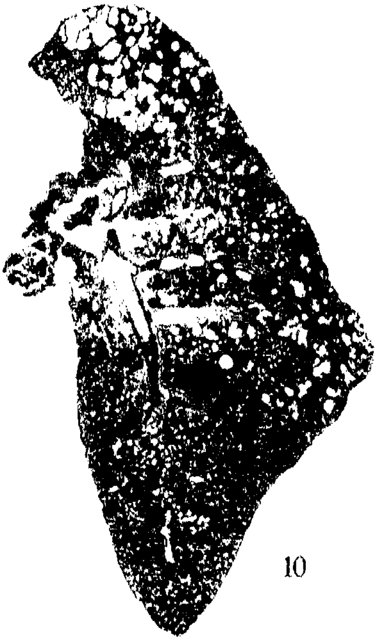

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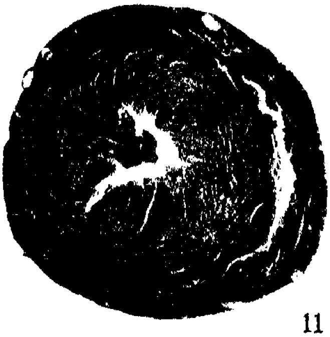
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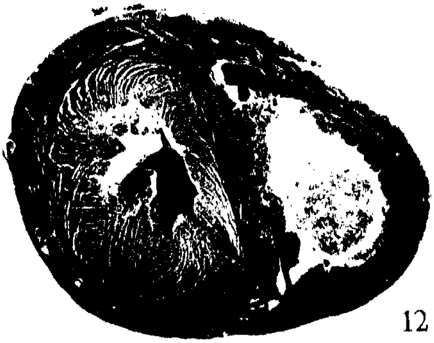
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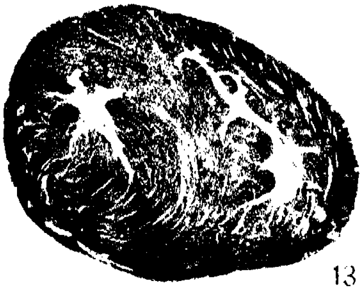
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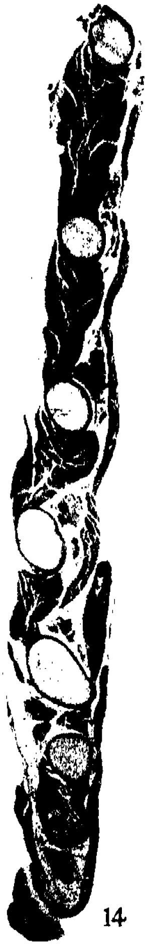
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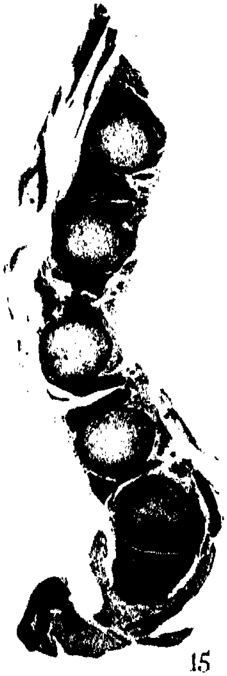
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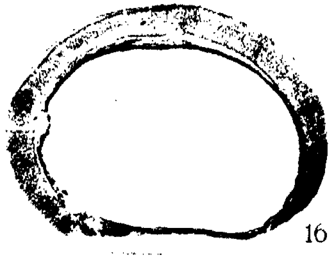
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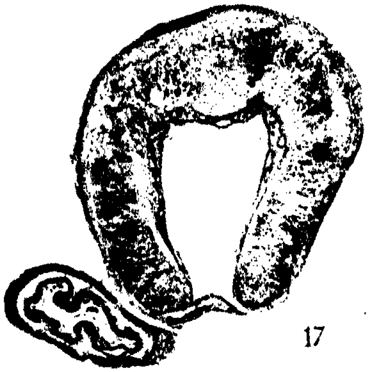
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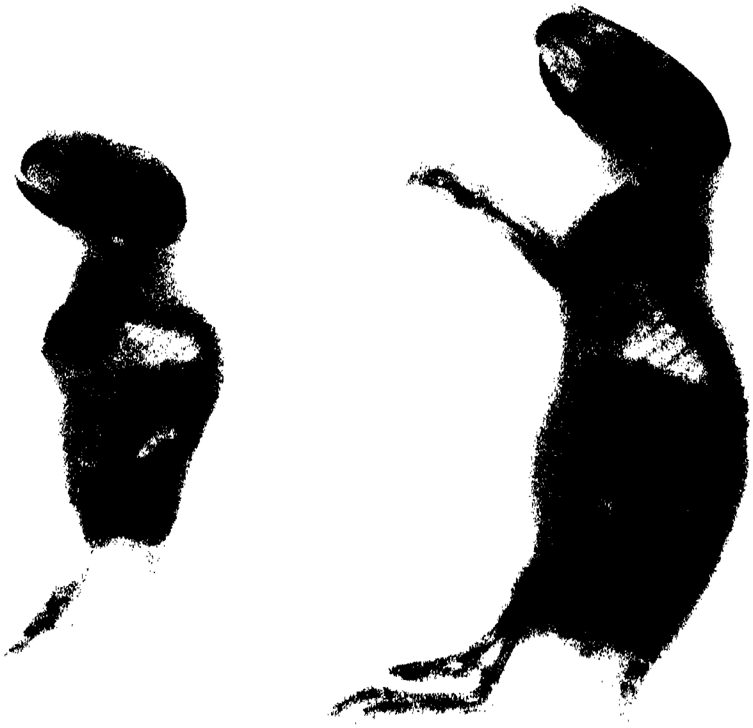
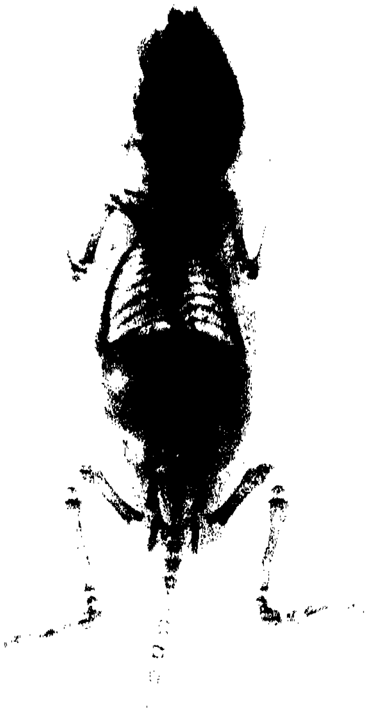


Fig. 18



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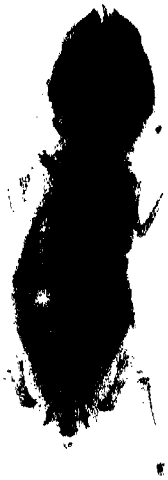

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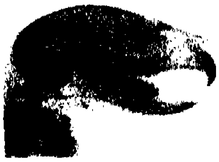

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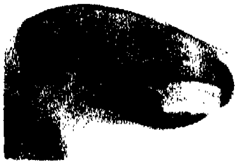

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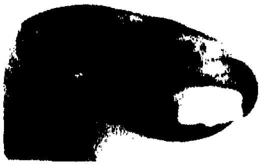

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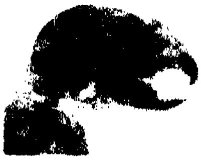

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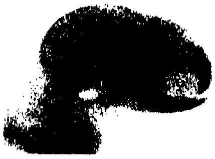

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virus has been obtained either from the salivary glands, or directly from the saliva, of any infective insect.

Viruses of the type causing Streak disease of Maize (Storey 1928) and Curly-top of Sugar beet (Bennett 1934, 1935) are not transmitted by insects immediately after feeding on the infected plants, but a so-called incubation period of the virus in the vector is necessary. Also these insects become infective for long periods, sometimes for the whole of their lives, after a single feeding on the source of infection, and the duration of their infectivity seems to depend, to some extent, on the length of this feeding. In these insects transmission is obviously not merely mechanical transfer, for it must be assumed that the virus is in the body of the vectors for some time before it is able to cause an infection, and possibly the insect acts as a kind of reservoir of virus from which successive infective doses can be withdrawn as long as the supply lasts.

Other viruses with similar properties, such as those causing Potato Leaf-roll (K. M. Smith 1933) and Aster yellows (Kunkel 1926), must also be included in this class, though their presence within the bodies of their vectors has not, so far, been demonstrated. Hartzell (1937) claims to have observed inclusion bodies in the cells of salivary glands of insects carrying Aster yellows, but this work requires confirmation as the objects described are very different from the inclusion bodies usually associated with plant viruses. This lack of information as to the presence of virus in particular organs of infective insects cannot be taken as evidence that it does not occur, for the detection of minute quantities of virus in the bodies of small insects presents many technical difficulties. On the other hand, for some viruses there has not only been complete failure to demonstrate the presence of the virus within the bodies of the vectors, but some of their properties appear to be in direct opposition to those which place Leaf-roll and Aster yellows viruses in the same class as Streak disease of Maize. Potato virus Y, Cucumber virus 1, Pea mosaic virus 2, and Hyoscyamus virus 3 can all be transmitted by their vectors within a few minutes of a short exposure to the source of infection, and the insects cease to be infective after a period of time not longer than the survival time of the virus in expressed sap. For these reasons, although such viruses are specific to their particular vectors, workers on Cucumber virus 1 (Doolittle and Walker 1928; Hoggan 1934) and Pea mosaic virus (Osborne 1937) have returned to the opinion that infection is caused by contamination of the stylets, and is a direct, mechanical inoculation.

Recently I have shown (Watson 1936) that Hy 3 virus exhibits some properties pertaining to its transmission by the aphid, *Myzus persicae*

(Sulz.), which do not seem to be explicable on the hypothesis of a simple, mechanical inoculation. The same type of behaviour has since (Mitchell 1937) been observed for Potato virus Y, which is similar to Hy 3 virus in most of its properties, and it is reasonable to suppose that other viruses of the same type may be transmitted in the same way.

The most unexpected result obtained with these two viruses was that the percentage of transmissions by individual aphides had its maximum value when the aphides were allowed to feed for only 2 or 3 min. on the source of the virus, and decreased rapidly with increasing feeding periods up to 1 hr. This observation cannot be explained on the hypothesis of simple mechanical transmission, for an aphid which has fed for 1 hr. on the infected plant should, other factors being equal, have at least as great, and probably a greater, chance of receiving virus contamination on its stylets than one which had fed for only 2 min.

In the experiments described (1936) all the aphides were starved for some hours before feeding on the source of infection, so as to ensure uniform feeding, and it was suggested that this might have some effect upon their efficiency as vectors. Further experiments have shown conclusively that the capacity of an aphid for causing infection can be greatly affected by such preliminary starving. The treatment of the aphid before and after exposure to the source of infection appears to be of far greater importance in determining their infectivity than the treatment accompanying the feeding itself. It has been the object of the present work to examine the effects of such starving treatments, and to determine how far the results could be reconciled with the existing hypothesis concerning the mechanism of transmission of Hy 3 and similar viruses. It was found that even if the aphides were prevented from feeding for only a short period of time before feeding on the infected plants, their infectivity was greatly increased. For such short periods it appeared preferable to refer to these aphides as fasting rather than starving. It will be shown, in fact, that there appear to be two distinct effects of abstention from food, only one of which can be attributed to starvation, in the strict sense of acute hunger.

METHODS

In all the experiments the sources of the virus were leaves detached from young tobacco plants, about three weeks from the date of their infection. The infectivity of each aphid was tested separately by allowing it to feed upon a healthy seedling tobacco plant, and sometimes on several such seedlings in succession. The general conditions of glasshouses, insectary, plants, aphid cultures, and technique, were essentially the same as have already been described (1936).

Short feeding periods of from 2–5 min. were measured from the time at which each aphid was observed to be quiescent and in the feeding position, except that if the number to be fed on infected leaves was too large for each individual to be observed separately, they were placed on the infected leaves in groups of about one dozen, and those individuals were used which were observed to be feeding at the end of 5 min. Previous results had shown that on the average this would allow each individual 3 min. "penetration time" (1936) and about 2 min. feeding. In optimum conditions aphides fed in this way gave results almost equal to those from more accurately timed feedings.

The following list of special terms has been used in the description of the experiments, for the sake of conciseness:

Infection Feeding—Feeding each aphid upon the source of infection. All aphides do not necessarily become infective after Infection Feeding.

Preliminary Fasting—Fasting before Infection Feeding.

Post-Infection Fasting—Fasting after Infection Feeding.

Intermediate Fasting—Fasting between two successive Infection Feedings given to the same aphid.

Final Fasting—Fasting after two successive Infection Feedings given to the same aphid.

Infection Trial—The test of infectivity of a single aphid by feeding on a single healthy plant.

In all experiments the effect of varying one or more of the following factors was investigated:

(1) Preliminary Fasting times.

(2) Infection Feeding times.

(3) Post-infection Fasting times.

(4) Time of feeding on the healthy plants.

In the first experiment a fifth factor, namely Time of Day, was also included.

The experiments were arranged on a factorial design in which the treatments consisted of all combinations of two or more of these variable factors. The factorial design was adopted because it not only enables the effect of varying a single factor to be measured with the greatest economy of labour and materials, but also enables any interactions between the different factors to be determined. Interaction between factors implies that the effect of one factor is dependent upon the intensity of another factor or factors.

As the treatments in all the experiments were the same kind, differences in the results being obtained by varying some factors, while keeping others constant, the same general procedure was followed in all. First a number of aphides, greatly in excess of the number required for the experiment, was collected from the food plants. The excess allowed for any injury or loss during the various stages of the experiment. These aphides were then either given an Infection Feeding at once, or given a constant period of Preliminary Fasting, or divided into groups, each group being given a different period of Preliminary Fasting. After this the groups, as they finished their respective fasting periods, could be given either a constant period of Infection Feeding, or (in experiments 1 and 2 only) divided into subgroups, each subgroup being given a different Infection Feeding time. The same process of subdivision was repeated for subsequent Fasting, Infection Feeding, or whatever variation of treatments was given, until the final subdivisions, in which each aphid had received the same combination of treatments. The experiments were all repeated a number of times, partly because it was not possible to treat a sufficiently large number of plants on a single occasion and partly to equalize the effects of temporary variation in uncontrolled conditions.

When individual aphides were given two or three infection trials, each aphid was numbered and the healthy plants on which it was fed were given a corresponding number, irrespective of the treatments involved. These aphides fasted in small, numbered tubes. If one of them died or was lost before the end of the experiment all the plants bearing its number were discarded. Extra plant series were started for each combination of treatments, to allow for this possible loss of material.

RESULTS

Experiment I. Effect of Preliminary Fasting, Infection Feeding time, and Time of Day.

This experiment was made to test the effect of Preliminary Fasting on the number of plants infected by individual aphides, which had been given varying Infection Feeding times. Also it was designed to show any possible effect of Time of Day on the numbers of infections obtained, for if such an effect existed it would affect the results of experiments in which it was necessary to compare infection trials made at different times of the day.

Treatments:

(1) Preliminary Fasting; None, 6 hr. (for afternoon cultures), 12 hr. (for morning and evening cultures).

(2) Infection Feeding—2 min., 15 min., 1 hr.

(3) Time of Day—9.30 a.m., 3.30 p.m., 9.30 p.m.

(4) Time on healthy plants—Constant—24 hr.

Five infection trials were made for each combination of trêatments and the whole was repeated on seven occasions at weekly intervals. The results are given in Table I.

TABLE I. EXPERIMENT I. EFFECT OF PRELIMINARY FASTING AND TIME OF DAY ON THE NUMBERS OF PLANTS INFECTED BY APHIDES AFTER VARYING INFECTION FEEDING TIMES. (35 INFECTION TRIALS FOR EACH OF 24 COMBINATIONS OF TREATMENTS)

	Periods of Infection Feeding								
	Preliminary Fasting				No Preliminary Fasting				
Time of Day	2 min.	15 min.	1 hr.	Total	2 min.	15 min.	1 hr.	Total	Total
9.30 a.m.	27	12	4	43	8	6	3	17	60
3.30 a.m.	21	10	4	35	5	5	6	16	51
9.30 p.m.	28	12	5	45	5	7	2	14	59
Total	76	34	13	123	18	18	11	47	170
Percentage infection (±3.15)	72	32	12	—	17	17	10	—	—

Preliminary Fasting had a very obvious effect in increasing the number of plants infected in a given number of trials, for more than twice as many infections were obtained after Preliminary Fasting as after continuous feeding. The magnitude of this effect, however, varied greatly with the length of the Infection Feeding time. When this was short the difference in performance between Preliminary Fasting and continuous feeding was nearly four times, but the difference decreased with increasing Infection Feeding times, and after 1 hr. there was no effect.

It was evident from this experiment that the results previously obtained (1936), in which the percentage infection decreased with increasing Infection Feeding time, depended upon the fact that the aphides had been given Preliminary Fasting, for in the present experiment the continuously fed aphides gave only a very slight decrease in percentage infection with increasing Infection Feeding time. Thus the suggestion put forward in 1936 to explain the results as they were then known, which was that the aphides developed seriological immunity to the virus in the first hour of Infection Feeding, is obviously untrue, for it would have to be assumed that the antibody was permanently present in unstarved aphides.

There were no regular differences in number of plants infected, corresponding to culturing at different times of the day. The number of infections obtained at the afternoon trials was slightly less than for the other times, but the difference was small and not significant.

Experiment II. Effect of Post-infection Fasting after varying periods of Infection Feeding

Previous experiments (1936) had shown that aphides could infect more than one healthy plant consecutively after only one Infection Feeding, but that increasing the period of time spent on the first of these healthy plants caused rapid decrease in the number of second plants infected. The present experiment was made to determine whether the infectivity of the aphides would be affected in the same, or in a different way, if a period of fasting was given instead of the time spent on the first healthy plant. No infections were obtained after starving for 24 hr., and the results are only given up to 12 hr.

Treatments:

(1) Preliminary Fasting—Constant 12 hr. (overnight).

(2) Infection Feeding—2 min., 15 min., 1 hr., 6 hr., 12 hr.

(3) Post-infection Fasting—2 min. (i.e. none, for this time was taken up in the "penetration time"), 15 min., 1 hr., 6 hr., 12 hr.

(4) Time on healthy plants—Constant—24 hr.

Six infection trials were made for each combination of treatments and the whole was repeated on ten occasions at weekly intervals. The results are given in Table II.

The numbers of plants infected decreased rapidly with increasing periods of Post-infection Fasting, falling to zero between 6–12 hr. There was no significant difference in the rates of fall between the different Infection Feeding times, though the relative rate of decrease seemed to be slower when the infection Feeding times were short. This was supported by the results of later experiments. On the total results the rate of loss of infectivity was not so rapid as in the Consecutive Infection experiments (1936), when the aphides were fed on healthy plants immediately after their Infection Feeding, but it was considerably faster than the inactivation rate of the virus *in vitro*, for sap from plants infected with Hy 3 virus is still slightly infective after four days at room temperature. These results will be discussed in connexion with the later experiments.

TABLE II. EXPERIMENT II. EFFECT OF VARYING PERIODS OF POST-INFECTION FASTING AFTER VARYING PERIODS OF INFECTION FEEDING. (60 INFECTION TRIALS FOR EACH COMBINATION OF TREATMENTS)

	Post-infection Fasting times						Percentage infection
Infection Feeding times	None	15 min.	1 hr.	6 hr.	12 hr.	Total	(± 3·32)
2 min.	39	31	15	7	1	93	31
15 min.	26	21	18	5	0	70	23
1 hr.	22	11	7	1	0	41	14
6 hr.	14	13	9	1	0	37	12
12 hr.	17	18	8	7	0	50	17
Total	118	94	57	21	1	291	—
Percentage infection (± 3·32)	39	31	19	7	0·3	—	—

The main effect of varying the Infection Feeding time was similar to that obtained in previous experiments, except that the period of minimum infectivity appeared to be rather more protracted. However, even in the 1936 experiment the rise in infectivity with increasing periods of Infection Feeding of from 1–6 hr. was not significant, though the increase from 1–12 hr. was so. Thus there is no disagreement between the two sets of results.

Experiment III. Effect of varying periods of Post-infection Fasting after varying periods of Preliminary Fasting

This experiment was designed to test the effect of different periods of Preliminary Fasting on the rate of decrease in the infectivity of the aphides due to varying periods of Post-infection Fasting.

Treatments:

(1) Preliminary Fasting—zero, 15 min., 1 hr., 6 hr.

(2) Infection Feeding—Constant—2 min.

(3) Post-infection Fasting—zero, 15 min., 1 hr., 6 hr.

(4) Time on healthy plants—Constant—24 hr.

Five infection trials were made for each combination of treatments and the whole experiment was repeated on ten occasions at weekly intervals. The results are given in Table III.

TABLE III. EXPERIMENT III. EFFECT OF VARYING PERIODS OF PRELIMINARY FASTING ON LOSS OF INFECTIVITY DUE TO POST-INFECTION FASTING. (50 INFECTION TRIALS FOR EACH COMBINATION OF TREATMENTS)

Preliminary Fasting times	Post-infection Fasting times					Percentage infection (± 2·4)
	None	15 min.	1 hr.	6 hr.	Total	
None	6	2	1	0	9	4·5
15 min.	18	11	6	1	36	18
1 hr.	27	15	7	0	49	24
6 hr.	32	22	9	2	65	32
Total	83	50	23	3	159	—
Percentage infection (± 2·4)	41	25	11	1·5	—	—

The main results from experiment III agree with those of previous experiments. Increasing periods of Preliminary Fasting caused increase in the numbers of plants infected, and increase in the Post-infection Fasting times caused increasing loss of infectivity. If the figures be considered in the form of curves it will be seen that the steepest part of the Preliminary Fasting curve lies between zero and 15 min. and the shallowest between 6–12 hr. This suggests that the effect of Preliminary Fasting began as soon as the aphides had finished feeding, for if there had been any appreciable lag, the increase up to 15 min. would have been incredibly fast.

The same is true to a less extent for the effects of Post-infection Fasting, for here the descending curve was also steepest for the shorter times, and the reduction in infectivity appeared to begin as soon as the insects had finished feeding. In this the type of inactivation seems to be different from that which occurs *in vitro*, for it is possible to detect only a slight loss of infectivity in extracted plant sap for the first few hours it is kept at room temperature. In the individual rates of decrease for Post-infection Fasting experiment III agrees with experiment II, for again there is a suggestion that the relative loss of infectivity is slower when Infection Feeding was preceded by Preliminary Fasting. The number of plants infected by the continuously fed aphides decreased by 66 % in the first 15 min., whereas the average decrease for all periods of Preliminary

Fasting was only 24 % in the first 15 min. No difference in the rates of fall could be detected between the three positive times of Preliminary Fasting.

It may also be noted that the infectivity loss in this experiment, the aphides having been fed before the Infection Feeding and fasted after it, is similar to that shown in the Consecutive Infection experiments described in the previous paper (1936), in which the aphides fasted before the Infection Feeding, but were fed on intermediate healthy plants immediately after it, and before their final infection trials. A comparison of the two sets of results is as follows:

1936 *Consecutive Infection experiments*	Times on intermediate healthy plants			
(Preliminary Fasting, 12 hr. Infection Feeding, 2 min.—12 hr.)	5 min.	15 min.	1 hr.	6 hr.
Percentage Infection:	15	6	1	0

Experiment III	Times of fasting after Infection Feeding			
(Preliminary Fasting, 0 Infection Feeding, 2 min.)	None	15 min.	1 hr.	6 hr.
Percentage Infection:	12	4	2	0

From these observations it is suggested that the relative rate of loss of infectivity in the aphides after Infection Feeding depends upon whether or not the aphides contain food during the time of inactivation of the virus, irrespective of the order or conditions in which the feeding treatments are given.

Experiment IV. Effect of fasting before and after two independent Infection Feedings

The experiments so far described were designed to show the effects of various treatments on the infectivity of the aphides after a single Infection Feeding. Later experiments were made to test whether similar effects would be obtained at subsequent Infection Feedings, or whether the fact that an aphid had once acquired and lost infectivity would affect its capacity to transmit. In the first of these experiments (experiment IV) each aphid was given two Infection Feeding periods of 2 min. each, separated by varying intervals of Intermediate Fasting. Periods of fasting were also given before the first and after the second Infection Feeding for two reasons. One was that the effect which was being examined might be dependent upon some particular condition of the aphides, that is to say, whether they had previously fasted or not, and the other was to provide a measure of the extent to which the Intermediate Fasting acted as a Post-infection Fasting to the first Infection Feeding and a Preliminary Fasting to the second Infection Feeding.

Treatments:

(1) Preliminary Fasting—zero, 1 hr., 6 hr., 12 hr.
(2) First Infection Feeding—Constant—2 min.
(3) Intermediate Fasting—zero, 15 min., 1 hr., 12 hr.
(4) Second Infection Feeding—Constant—2 min.
(5) Final (Post-infection) Fasting—zero, 15 min., 1 hr., 12 hr.
(6) Time on Healthy Plants—Constant—24 hr.

(For zero Intermediate Fasting only one 2 min. period of Infection Feeding was given, since to have given two successive feeding periods would have introduced a factor of increased Infection Feeding period, which would have destroyed the value of this treatment as a control to the others.) Three infection trials were made for each of the sixty-four combinations of treatments and the whole was repeated on ten occasions at weekly intervals. The results are given in Table IV.

For comparison with the results of previous experiments in which similar treatments were given, the main effects are shown in Table V as percentages of total number of plants infected out of the total number of trials.

The main effects, for varying periods of Preliminary, Intermediate and Final Fastings respectively, are all significant at the 1 % level, though the individual differences are not always significant. For instance, there is a slight increase from 6–12 hr. Preliminary Fasting, but this difference is not significant, though the increase from zero to 12 hr. is highly significant. The effect of each factor will be described separately.

(1) *Preliminary Fasting*. These results agreed very well with those of experiments I and III, for the number of plants infected increased with increasing periods of Preliminary Fasting according to expectation. The differences in the other treatments of the aphides make it difficult to compare the numerical results exactly in the three experiments, but, as in experiment III, a large increase occurred in the first hour of Fasting, and comparatively little was gained, per hour, by continuing to starve for much longer periods.

(2) *Final Fasting*. It is convenient to consider the effects of Final Fasting before those of Intermediate Fasting, because it is believed that an

TABLE IV. EXPERIMENT IV. EFFECT OF VARYING PERIODS OF FASTING BEFORE AND AFTER TWO INDEPENDENT INFECTION FEEDINGS. (30 INFECTION TRIALS FOR EACH OF 64 COMBINATIONS OF TREATMENTS)

Note. The total number of infections obtained for the varying periods of Preliminary Fasting are shown by the grand totals of the four sections which make up the main body of the table. The totals for Intermediate and Final Fastings, for all periods of Preliminary Fasting, are given in the subsidiary table.

Preliminary Fasting	Intermediate Fasting	Final Fasting times				
		None	15 min.	1 hr.	12 hr.	Total
None	None	9	3	1	0	13
,,	15 min.	10	10	4	1	25
,,	1 hr.	16	13	5	2	36
,,	12 hr.	12	10	10	0	32
	Total	47	36	20	3	106
1 hr.	None	16	13	8	1	38
,,	15 min.	13	11	10	0	34
,,	1 hr.	20	15	10	1	46
,,	12 hr.	13	9	6	2	30
	Total	62	48	34	4	148
6 hr.	None	22	9	9	2	42
,,	15 min.	17	16	7	0	40
,,	1 hr.	19	14	15	1	49
,,	12 hr.	16	11	3	1	31
	Total	74	50	34	4	162
12 hr.	None	18	13	8	0	39
,,	15 min.	17	12	9	0	38
,,	1 hr.	24	22	11	2	59
,,	12 hr.	19	13	5	0	37
	Total	78	60	33	2	173

Subsidiary Table for all Preliminary Fasting times

Intermediate Fasting times	Final Fasting times				
	None	15 min.	1 hr.	12 hr.	Total
None	65	38	26	3	132
15 min.	57	49	30	1	137
1 hr.	79	64	41	6	190
12 hr.	60	43	24	1	128
Total	261	194	121	11	587

effect equivalent to the Final Fasting effect is involved in the production of the Intermediate Fasting results.

TABLE V. EXPERIMENT IV. PERCENTAGE OF INFECTIONS OBTAINED BY FASTING BEFORE AND AFTER TWO INDEPENDENT INFECTION FEEDINGS

	Times of Fasting					Standard error
	None	15 min.	1 hr.	6 hr.	12 hr.	
(1) Preliminary Fasting	22·1	—	30·4	33·8	36·0	± 2·74
(2) Intermediate Fasting	27·5	28·5	39·6	—	26·7	± 3·14
(3) Final Fasting	54·4	40·4	25·2	—	2·3	± 3·97

As in experiment III and experiment II (for short periods of Infection Feeding) fasting after Infection Feeding caused rapid decrease in the infectivity of the aphides. Very few plants were infected after 12 hr. fasting, though the fact that even a small number of the aphides which had been given Preliminary Fasting were still infective after 12 hr. is of interest, for in experiment III 6 hr. fasting was sufficient to reduce infectivity to a very low level. This may indicate that, after Preliminary Fasting, inactivation of the virus is rapid at first, but that which is active after 6 hr. is not further inactivated so long as the aphid continues to fast, except presumably by normal deterioration such as occurs *in vitro*. Numerical comparisons between the three experiments are again difficult because of the differences in treatments and separation in time, but if results for aphides which received approximately similar treatments are selected from each experiment the effects of Post-infection Fasting are very similar. Table VI shows the numbers of plants infected in three experiments, by aphides which had been given from 6–12 hr. Preliminary Fasting followed by 2 min. Infection Feeding, and varying periods of Post-infection Fasting.

TABLE VI. COMPARISON BETWEEN EXPERIMENTS II, III AND IV FOR EFFECTS OF POST-INFECTION FASTING

		Times of Post-infection Fasting				
		Zero	15 min.	1 hr.	6 hr.	12 hr.
Percentage infection	Exp. II	66	50	25	11·0	1·7
	Exp. III	64	44	18	4	—
	Exp. IV (for one Infection Feeding)	66	35	28	—	3·3

Although experiments III and IV failed independently to give significant interaction between the treatments, there was agreement between them that the relative rates of loss of infectivity were not the same for all Preliminary Fasting treatments. Probably the absence of the significance is due to the fact that in both experiments the main difference lies between zero and all positive times of Preliminary Fasting, and the figures for zero Preliminary Fasting were very small in both experiments. If the section of experiment IV in which the aphides were given only one Infection Feeding is compared with experiment III, in which the aphides received almost identical treatments, it can be seen that the results agree closely as to the difference in relative rate of decrease between zero, and the mean of all positive Preliminary Fasting times. These rates of decrease, expressed as percentages of the initial aphid infectivity, are given in Table VII.

TABLE VII. COMPARISON BETWEEN EXPERIMENTS III AND IV FOR EFFECTS OF POST-INFECTION FASTING

Percentage of initial infectivity remaining after Post-infection Fasting times

		Times of Fasting			
		15 min.	1 hr.	6 hr.	12 hr.
Exp. III	Zero Preliminary Fasting	33	15	0	—
	Mean of all positive Preliminary Fasting times	62	29	4	—
Exp. IV (one Infection Feeding)	Zero Preliminary Fasting	33	10	—	0
	Mean of all positive Preliminary Fasting times	63	44	—	5

Intermediate Fasting. For Intermediate Fasting periods of from zero to 1 hr. the total number of plants infected was increased from 55 to 79 %. For periods of from 1–12 hr. Intermediate Fasting the percentage decreased from 79 to 53.

If the Intermediate Fasting effects were caused by the summation of Post-infection Fasting to the first Infection Feeding, and Preliminary Fasting to the second Infection Feeding, an increase for the shorter periods of Intermediate Fasting would be expected, for aphid infectivity is more rapidly increased by Preliminary Fasting than it is decreased by Post-infection Fasting. However, a simple summation of these two effects should give a much greater increase for the first hour than was actually obtained, and the subsequent decrease should be very slight as the Preliminary Fasting would, by this time, be giving its maximum effect.

From these considerations it seems that either there was some reduction in the capacity of the aphides to become infective after the second Infection Feeding as compared with the first, or else the effect of fasting before a second Infection Feeding was not the same as that of fasting before a first.

To determine if either, or both, of these possibilities was correct it was necessary to find out how much of the total infectivity of an aphid after two Infection Feedings was obtained at the second Infection Feeding. This was done by estimating the infectivity of the aphides after their first Infection Feedings and subtracting these values from the figures actually obtained after two Infection Feedings.

The different steps, and the results, of these calculations are tabulated in Table VIII.

The infectivity of the aphides after the second Infection Feeding was calculated for all combinations of Preliminary and Intermediate Fasting treatments separately, but the Final Fasting results were used *en bloc*. These could not affect the initial infectivity of the aphides, and the error due to variation in the rate of decrease for Final Fasting, with different periods of Intermediate Fasting, was more than compensated for by the increase in the number of observations.

A measure of the infectivity of the aphides after a single Infection Feeding (for all Final Fasting times) was obtained from the results for zero Intermediate Fasting, these aphides having been given only one Infection Feeding. These observations had been obtained for zero, 1, 6, 12 hr. fasting, but to compare the effects of Preliminary and Intermediate Starving directly, it was necessary that the same times should be considered for both treatments. As no value had been obtained for 15 min. Preliminary Fasting this was calculated from the results of experiment III where 15 min. Preliminary Fasting gave a value rather above the mean between those for zero and 1 hr. On this analogy 15 min. Preliminary Fasting would, in experiment IV, have given a value rather above the mean of 13 and 38 (see Table IV), or about 26 (see Table VIII). Furthermore, instead of the observed values for 12 hr. Preliminary Fasting (for zero Intermediate Starving) a rough mean between 6 and 12 hr. was used, for the total figures, as well as previous results, show that the reduction obtained for from 6–12 hr. Fasting could not have been a real effect.

As there were no observations for 15 min. Preliminary Fasting in experiment IV the infectivity of the aphides after the second Infection Feeding could only be estimated for zero, 1, and 12 hr. Preliminary Fasting. The loss in infectivity due to starving after the first Infection Feeding was estimated from the rates of Final Fasting for zero Intermediate Fasting. It had previously been shown that no difference in rate of decrease existed between zero and all positive periods of Preliminary Fasting, therefore the rates shown in Table VII (for experiment IV) were used.

The infectivity of the aphides after the first Infection Feeding was then calculated for each combination of Preliminary and Intermediate Fasting treatments, and subtraction of these values from the actual number of infections obtained after two Infection Feedings, for the same combinations of treatments, gave an estimate of the infectivity which the aphid had acquired at the second feeding.

TABLE VIII. EXPERIMENT IV. COMPARISONS BETWEEN THE INFECTIVITY OF APHIDES AFTER A FIRST INFECTION FEEDING AND THEIR INFECTIVITY AFTER A SECOND INFECTION FEEDING, AS ESTIMATED FROM THE KNOWN CONSTANT BEHAVIOUR AT A SINGLE FEEDING

(Actual figures obtained from Table IV, percentage figures from Table VII)

Infectivity after first Infection Feeding	Times of Intermediate Starving	Percentage decrease for fasting after Infection Feeding	Infectivity remaining from first feeding after Intermediate Fasting	Observed infectivity after two Infection Feedings	Calculated infectivity after second Infection Feeding	Infectivity after a single Infection Feeding for corresponding Preliminary Starving times
			No Preliminary Fasting			
13	None	100	13	13	—	13
—	15 min.	33	4	25	21	26*
—	1 hr.	11	1	36	35	38
—	12 hr.	0	0	32	32	40
			One hour Preliminary Fasting			
38	None	100	38	38	—	13
—	15 min.	63	24	34	10	26*
—	1 hr.	44	17	46	29	38
—	12 hr.	5	2	30	28	40
			Twelve hours Preliminary Fasting			
40	None	100	40	39	—	13
—	15 min.	63	25	38	13	26*
—	1 hr.	44	18	59	41	38
—	12 hr.	5	2	37	35	40

* Calculated value for 15 min. Preliminary Fasting.

It could not be expected that figures estimated in this way would be perfectly consistent, for the observed individual results depart in several places from the ideal curves suggested by their totals. For instance, for 1 and 12 hr. Preliminary Fasting the results for 15 min. Intermediate Fasting are below the results for zero Intermediate Fasting, and it is unlikely that this is a real effect. The indications, however, are that the aphid rarely acquires so much infectivity at the second feeding as it did at the first. This is particularly noticeable for zero and 1 hr. Preliminary Fasting, but for 12 hr. the calculated values for infectivity after the second Infection Feeding would have been very close to those which were estimated for a single feeding if the observed 15 min. value were according to expectation. The calculated values show rapid increase for increasing periods of Intermediate Fasting up to 1 hr., similar to the increase caused by Preliminary Fasting before a first Infection Feeding.

A possible explanation of these results is that Intermediate Fasting does have the same kind of effect as Preliminary Fasting, but that the aphides are discouraged from feeding by the successive changes from plant to plant, and by the continuous feeding on tobacco plants which are not one of their natural foods. This is supported by the fact that when they are in a starving condition, i.e. after two long periods of fasting with only very short feeding periods between, their second feeding gave results almost equal to the first. The effect of feeding on tobacco plants seems to correspond with what in higher animals would be called loss of appetite, and is probably the result of a general depression in the natural activity of the aphides, which after prolonged fasting is counteracted by an increase of feeding activity stimulated by starvation.

Experiment V. Comparison between infections obtained from aphides after feeding on Tobacco plants with those taken from Turnips

The suggestion that the aphides are less successful as vectors if their feeding activity is discouraged by continuous feeding on tobacco plants was tested by a simple experiment in which aphides reared on the food plants (Turnip) were compared in a series of infectivity trials with aphides which had previously fed for 24 hr. on young tobacco plants.

Treatments:

(1) Aphides taken straight from food plants *v.* aphides after 24 hr. on Tobacco plants.

(2) Infection Feeding—2 min. on Tobacco leaves.

(3) Feeding Time on healthy plants—Constant—24 hr.

Twelve infection trials were made for each treatment on each occasion and the whole was repeated in six successive weeks.

As no Preliminary Fasting was given to the aphides few infections were obtained. In all 19 % of the total number of plants were infected by the aphides from the food plants, and only 9 % by the aphides from tobacco; this difference was not significant, though it tends to support the observations made in the previous experiment.

Experiment VI. Effect of varying times on the healthy plants and various Fasting treatments before and after three independent infection trials

In the Consecutive Infection experiments described in the previous paper (1936), it was shown that aphides vary individually in their efficiency as vectors after a single Infection Feeding. Experiment VI was originally designed to determine whether their capacities for infection would remain constant over a succession of infection trials, and whether their efficiency was a permanent or temporary attribute of the different individuals. The aphides were therefore given three successive infection trials each, the time between the trials being varied by varying the time which they were allowed to feed on the different healthy plants.

The scope of the experiment was later extended to include the effect of varying Preliminary Fasting treatments on the constancy, or otherwise, of the aphides' efficiency as vectors. With this object the experiment was divided into three parts, so that the same series of infection trials were carried out on different occasions using aphides which had been differently treated in respect of Preliminary Fasting.

Part 1. *Plants infected at three successive trials by aphides which had no Preliminary Fasting*

Treatments:

(1) First Infection Feeding—Constant—2 min.
(2) First Feeding on healthy plants—15 min., 1 hr., 12 hr., 24 hr.
(3) Second Infection Feeding—Constant—2 min.
(4) Second feeding on healthy plants—15 min., 1 hr., 12 hr., 24 hr.
(5) Third Infection Feeding—Constant—2 min.
(6) Third feeding on healthy plants—Constant—24 hr.

Each combination of treatments was repeated three times on each occasion and the whole was repeated on five occasions in successive weeks. The results are given in Table IX.

The total number of plants infected at each successive trial decreases although the same treatments were given at each feeding. This is additional evidence in support of the suggestion that the efficiency of the aphides as vectors is affected by loss of appetite due to continuous handling, or continuous feeding on tobacco plants, or even by increasing age, for some treatments required an interval of 48 hr. between the first trial and the third. For individual treatment effects see below.

TABLE IX. EXPERIMENT VI, PART 1. EFFECT OF VARYING FEEDING TIMES ON HEALTHY PLANTS AT THREE SUCCESSIVE INFECTION TRIALS. NO PRELIMINARY FASTING

Time on first healthy plants	Time on second healthy plants														
	15 min.			1 hr.			12 hr.			24 hr.			Totals		
	1st	2nd	3rd*	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
15 min.	0	3	5	3	4	1	4	0	1	1	2	0	8	9	7
1 hr.	3	2	4	2	2	2	5	2	0	4	3	0	14	9	6
12 hr.	3	4	4	2	0	1	3	3	5	5	2	3	13	9	13
24 hr.	5	2	2	4	1	1	5	2	3	8	5	2	22	10	8
Totals	11	11	15	11	7	5	17	7	9	18	12	5	57	37	34

* 1st, 2nd, 3rd. Plants infected by the same aphides at successive infection trials.

Part 2. *Plants infected at three successive infection trials by aphides which fasted before the first Infection Feeding only*

Treatments. As in part 1 except that the first Infection Feeding was preceded by 12 hr. Preliminary Fasting. Repetitions, and occasions as in part 1. The results are given in Table X.

A much larger number of plants was infected at the first infection trial than at the other two, because these aphides were given Preliminary Fasting. The numbers of plants infected at the third trial was slightly less than at the second trial. This is in agreement with the results for part 1 of the experiment.

Individual treatment results for parts 1 and 2. Experiment VI

In addition to the effects of Preliminary Fasting and continuous transference to tobacco plants, the number of plants infected varied with the times for which the aphides were allowed to feed on the successive healthy plants. Owing to the small number of observations for each combination of treatments there were large standard errors and only very large differences were found to be significant, but the results were in agreement with those of previous experiments and it seemed improbable that any further information would be gained if the series were continued.

It has been shown previously (1936) that increasing the period for which aphides were allowed to feed on healthy plants immediately after Infection

Feeding caused a small but significant increase in the number of infections obtained. In experiment VI one effect of varying the feeding times on the healthy plants appeared to be similar to this, though the only significant difference was an increase of from 8 infections for 15 min. feeding, to 22 infections for 24 hr. feeding on the first healthy plants in part 1. In part 2 there was an increase from 30 infections for 15 min. feeding, to 38 infections for 12 hr. feeding on the first healthy plants, and an increase of from 11 infections for 15 min. feeding, to 21 infections for 12 hr. feeding on the second healthy plants. Both showed an unexpected decrease for 24 hr. feeding, but as a plant once infected cannot, presumably, lose its infection again, this cannot be a real effect of feeding time on the healthy plants.

TABLE X. EXPERIMENT VI, PART 2. EFFECT OF VARYING FEEDING TIMES ON HEALTHY PLANTS AT THREE SUCCESSIVE INFECTION TRIALS. PRELIMINARY FASTING OF 12 HOURS PRECEDING THE FIRST INFECTION TRIAL

Time on first healthy plants	Time on second healthy plants												Totals		
	15 min.			1 hr.			12 hr.			24 hr.					
	1st	2nd	3rd*	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
15 min.	8	2	4	8	6	3	6	9	2	8	7	1	30	24	10
1 hr.	8	4	6	8	3	3	5	4	1	7	2	0	28	13	10
12 hr.	8	3	6	12	1	1	8	4	4	10	2	2	38	10	13
24 hr.	7	2	4	10	0	2	6	4	2	7	1	2	30	7	10
Totals	31	11	20	38	10	9	25	21	9	32	12	5	126	54	43

* 1st, 2nd, 3rd. Numbers of plants infected by the same aphides at successive infection trials.

The numbers of infections obtained at the second and third infection trials were also affected by the time for which the aphides had fed upon the previous healthy plants. Thus an increase in the feeding time on the second healthy plants in part 1, of from 15 min. to 1 hr., caused the number of infections obtained at the third infection trial to drop from 15 to 5. In part 2 the increase of from 15 min. to 24 hr. feeding on the first healthy plants caused a decrease of from 24 to 7 plants infected at the second trial, and a similar increase in the feeding time on the second healthy plants caused a decrease from 20 to 5 in the plants infected at the third infection trial.

In this effect experiment VI agrees with experiment IV, for it was found then that increasing the interval of time between the two Infection Feedings given to each aphid caused a decrease in the infectivity of the aphides after the second Infection Feeding. In both experiments the apparent decrease was really caused by the fact that when the time between the

Infection Feedings was short, the infectivity at the second feeding was increased by virus remaining from the first feeding, but if the time, and treatments, between the two feedings were sufficient for this infectivity to have been lost, then the infectivity from the second feeding was generally lower than that from the first.

Part 3. *Plants infected at three successive infection trials; by aphides which fasted before every Infection Feeding; and comparison between these results and those obtained after similar feedings on healthy plants*

As the effect of varying the times of feeding on healthy plants between Infection Feedings had been obtained in the first two parts of Experiment V, the third part was arranged simply as a test of the individual performances of aphides at three successive infection trials separated by constant periods of fasting, but as an additional test for the effect of continuous feeding on tobacco plants in producing decreased numbers of infection, "mock" infection trials were made with aphides which were, for the first one or two trials in each series, given feeding and fasting treatments on healthy plants only, followed by a single infection trial to determine their infectivity. Thus there were three series of treatments, which have been called series A, B and C. The results from series A only were used for comparison with the totals from parts 1 and 2 of experiment VI.

Treatments:

(1) Preliminary Fasting—Constant—6 hr.

(2) First Infection Feeding—series A only—2 min.

(3) Feeding time on healthy plants—Constant—18 hr.

(4) Fasting—Constant—6 hr.

(5) Second Infection Feeding—series A and B only—2 min.

(6) Feeding time on healthy plants—Constant—18 hr. (Final feeding for series B.)

(7) Fasting—Constant—6 hr.

(8) Third Infection Feeding—A and C only—2 min.

(9) Time on healthy plants—Constant—18 hr.

Each treatment was repeated on 15 plants for each repetition of the experiment, that is to say at each repetition there were 15 plants for each of the three Infection Feedings in series A; two sets of 15 healthy plants in series B of which only the second set received infection-fed aphides, and three sets in series C in which only the third set received infection-fed aphides. The whole was repeated on six occasions at weekly intervals. The results are given in Table XII.

There was a rather large standard error, partly because of the irregular arrangement of the experiment, but on the whole the results agreed with those of the previous experiments in that the number of infections obtained at the end of long successions of treatments were reduced, even though the previous treatments did not include feeding on infected leaves. Thus the reduction was not due to the fact of the aphides having been infective at a previous trial, but merely to loss of feeding activity or "appetite", caused by the artificial conditions.

TABLE XI. INFECTIONS OBTAINED AFTER SUCCESSIVE TRANSFERS OF APHIDES TO TOBACCO PLANTS WITH AND WITHOUT INFECTION FEEDING BETWEEN THE FIRST AND SECOND TRIALS. FASTING PERIODS WERE GIVEN BEFORE EACH TRIAL

	Series A			*Series B*	*Series C*	
	Three transfers with Infection Feeding between each			Two transfers with final Infection Feeding only	Three transfers with final Infection Feeding only	Standard error
	1st	2nd	3rd	2nd	3rd	
Plants infected out of 90	57	66	45	46	50	± 4·602
Percentage infection	63·3	73·3	50·0	51·1	55·5	± 6·91

The aphides in series A infected a much greater proportion of the plants used at the second and third trials than did those for the corresponding treatments in parts 1 and 2 of experiment VI. This increase was caused by the fasting periods which were given to these aphides before each Infection Feeding.

Experiment VI continued. Variation in the capacities of aphides for causing infection at successive infection trials

The efficiency of an aphid cannot be determined from the results of a single Infection Feeding, for its success or failure may depend upon some outside factor, but if it is successful at two independent feedings, while another aphid fails at both, it may possibly be said that the first aphid is a better vector than the second, and the more occasions on which the first aphid is successful and the second unsuccessful, the more probability there is that a fundamental difference in infective capacity exists between the two aphides.

The aphides from the three parts of experiment VI can be classified according to their success or failure at the three Infection Feedings which they were given. Table XII shows such a classification giving the percentage of the total number of aphides used in each part of the experiment, which fall into each class, i.e. those failing to give infection at every trial, those giving infection at the first trial only, and so on. The number of aphides which fell into each class was greatly affected by the different Preliminary Fasting treatments. For instance, in part 1 60 % of the aphides failed to give an infection at all, while in part 2 only 30 % of the aphides failed completely, but a relatively large number gave infection at the first trial only, because their efficiency was increased by fasting before the first Infection Feeding. A high proportion of these aphides was also successful at the second trial because, when the time between the trials was short, the effect of the Preliminary Fasting was still in evidence, but the number of aphides successful at all three trials was very small. On the other hand, when Preliminary Fasting was given at each infection trial, 33 % of the aphides were successful at all three trials and only 11 % failed to give any infections. As only one aphid in part 3 gave infection at the last trial only it is unlikely that many of the 11 % which were always unsuccessful could ever have been persuaded to infect, unless there is some other condition to which they could have responded.

TABLE XII

Classes of possible Performance at each infection trial			Percentage of aphides in each class		
1st	2nd	3rd	Part 1	Part 2	Part 3
0	0	0	59·6	30·8	11·1
+	0	0	15·0	32·1	7·8
0	+	0	7·5	8·3	11·1
0	0	+	6·7	6·2	1·1
+	+	0	3·3	10·8	16·7
+	0	+	2·9	8·3	5·5
+	+	+	2·5	1·7	33·3
0	+	+	2·0	1·7	12·2

From the percentage figures in Table XII three measures of the efficiency of each aphid can be obtained for each part of the experiment. By comparing their performance at their first and second trials, with those for the first and third, and second and third trials, it can be determined whether any of the individuals consistently differed from the others. If the highest proportion of later infections came from aphides which had previously given infection, then these aphides can be said to have been more efficient vectors than the rest, but if the later infections were obtained equally from aphides which had been successful and unsuccessful on the previous plants, then their success, or lack of it, was due to some other factor than inherent ability.

For example, from Table XII it can be seen that 76·3 % of the aphides used in part 1 of the experiment gave no infection at their first Infection Feeding, and of these 9·5 % gave infection at the second Infection Feeding, so that 12·5 % of the aphides which did not infect the first plant, infected the second. On the other hand, 23·7 % of the aphides gave infection at their first trial, and 5·8 % infected at both their first and their second trials, so that 24·4 % of the aphides which gave infection at their first trial also gave infection at their second. This is nearly twice the percentage of aphides which gave infection on the second plant after failing on the first, suggesting that some of the aphides were consistently more efficient vectors than others.

TABLE XIII. PERCENTAGES* OF APHIDES WHICH GAVE INFECTION AFTER PREVIOUSLY HAVING GIVEN INFECTION, COMPARED WITH THOSE WHICH FAILED AT THE PREVIOUS TRIAL; FOR THE THREE PARTS OF EXPERIMENT VI

Performance at first trial	Percentage which gave infection at second trial	Performance at second trial	Percentage which gave infection at third trial	Performance at first trial	Percentage which gave infection at third trial
Part 1. No Preliminary Fasting					
0	12·4	0	11·8	0	12·5
+	24·5	+	33·3	+	23·4
Part 2. Preliminary Fasting at first trial only					
0	21·3	0	18·2	0	17·0
+	24·5	+	17·4	+	18·9
Part 3. Preliminary Fasting at every infection trial					
0	65·9	0	25·5	0	37·3
+	78·9	+	62·1	+	61·5

Table XIII gives a list of the percentages of aphides which infected the second plant after infecting the first, compared with those which infected the second plant after failing to infect the first. The percentages are given for each of the three possible comparisons between different plants in each series, for each part of the experiment.

The remaining comparisons in part 1 are in agreement with the first. At the second and third trials the aphides which had already given one infection had a greater chance of giving a subsequent infection than those which had not. That is to say that in part 1, one section of the aphides showed themselves to be consistently more efficient vectors than the rest.

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* Calculated from the actual figures and not from the percentage figures given in Table XII.

For part 2 no such result was obtained. There was no difference in the percentage of plants infected at later trials whether the aphides had been successful at a previous trial or not. This result was consistent for all possible comparisons between the different infection trials in part 2, so that none of these aphides showed superior efficiency as vectors. For part 3, however, the results again clearly indicate that some of the aphides were more successful vectors than others throughout the whole series of trials.

Thus, when the aphides fasted before each Infection Feeding, some of them gave evidence of consistently higher ability to carry infection than others. If no Preliminary Fasting were given, there were still some aphides which showed high efficiency, and others did not, but when both these treatments were given in turn no aphides showed any consistent superiority.

It appears that aphides which were successful in the feeding condition were not necessarily those which responded to Preliminary Fasting, and aphides which gave high infectivity after Preliminary Fasting would not necessarily have been more than normally successful without it.

DISCUSSION AND SUMMARY

It has been shown that *Myzus persicae* is much more likely to transmit Hy 3 virus if made to fast immediately before being fed on the source of infection. Its efficiency as a vector increases rapidly during the first hour of fasting, but long periods of starving give but little further increase in infectivity.

The increased efficiency of the aphides as vectors induced by Preliminary Fasting decreases as the Infection Feeding period is increased, and after one hour's feeding on the infected leaves, Preliminary Fasted aphides give no more infection than do continuously fed aphides.

Infectivity is lost by the aphides after the cessation of Infection Feeding, and the rate of loss is probably more rapid than the rate at which the virus deteriorates *in vitro*. This loss of infectivity is complete in about one hour if the aphides do not fast before Infection Feeding, or if they are fed on an intermediate healthy plant between the Infection Feeding and the infection trial, but if the aphides fast both before and after the Infection Feeding the relative rate of loss of infectivity is slower and a few insects may remain infective for many hours.

The infectivity of an aphid is greater after two or more successive Infection Feedings of equal length unless the treatment given between the feedings is such that all infectivity acquired at the earlier feeding is lost in one of the above ways. The infectivity actually acquired at a second or third Infection Feeding is, however, generally less than that acquired at the previous feedings. This difference is least noticeable when the aphides have been given long fasting periods and only very short feeding periods, and there is some evidence that it is due to loss of appetite caused by continuous feeding on unsuitable food plants, or by slight damage in successive transfers.

If the same Preliminary Fasting treatments are given over a succession of infection trials, some aphides show consistently greater ability to transmit the virus than others, but if the Preliminary Fasting treatments are varied, so that aphides fast and feed continuously, at alternate infection trials, there is no evidence of consistent superiority for any individuals. This indicates that the infectivity of the aphides is affected by two factors, both connected with the fasting condition, but only one of which, and probably the least effective, can be that of "appetite". If "appetite" can be considered to be increase or decrease in the natural feeding activity of the insects, then the main effect of Preliminary Fasting is concerned with some distinct and independent factor.

It is possible to explain these results if it is assumed that, during the process of transmission from one plant to another, the virus comes into contact with some substance which partially or wholly destroys its infectivity.

The fact that the aphides are more efficient after fasting than when continuously fed, suggests that the variation in efficiency may be connected with variation in the production of enzymes, for in most insects enzyme production, at least from the stomach, ceases during starvation.

The chief proteolytic enzyme found in the stomach of insects is trypsin (Wigglesworth 1934), and insect trypsin appears to be similar to pancreatic trypsin. Enzymes of the pepsin type acting in very acid media have not been found in insects.

The virus is precipitated from plant extracts by protein precipitants, and is antigenically active, which indicates that it is associated with a considerable amount of protein. Furthermore the virus preparations, whether fresh plant extracts or after concentration by precipitation with ammonium sulphate, are instantly reduced in infectivity by merely mixing with trypsin. Incubation with carbohydrases such as amylase or invertase has no effect upon it.

If the quantity of trypsin added to a solution of virus is not sufficient for immediate complete loss of infectivity, then even after incubation for several hours at 38° C. there will be very little further loss of infectivity. This is reminiscent of the effect noticed in the transmission experiments, that if fasting insects are given a very short Infection Feeding, and immediately fasted again, a few will retain their infectivity for many hours, whereas feeding insects lose all their infectivity in a comparatively short time.

These facts suggest that reduction in the infectivity of the virus during transmission by feeding insects may be caused by contact with trypsin.

There is no evidence to show how the virus could come into contact with the trypsin. It is unlikely that trypsin is introduced into the plant with the saliva, for the salivary glands of such phytophagous insects as have been studied have been found to secrete only enzymes which attack carbohydrates, usually amylase and occasionally invertase (Wigglesworth 1934), and these apparently do not affect the virus. It might conceivably be introduced into the plant by regurgitation from the stomach, but if regurgitation takes place it is equally probable that the virus itself is regurgitated after having been in contact with the trypsin. Regurgitation appears unlikely, for it would have to be effected by opening of the oesophageal valve, a function for which no special muscles appear to exist, and any accidental leakage from the stomach would prevent efficient working of the suction pump by which the aphid obtains its food. On the other hand there is equal difficulty in the supposition that the virus passes through the stomach wall and reaches the salivary glands, for not only is the complete process of transmission unaccountably fast for such a series of movements, but also the virus is non-dializable and presumably could not passively diffuse through a semi-permeable membrane such as is formed by the gut wall of animals, and so far no lesions have been observed which indicate that it does so by any other means.

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Cytochrome oxidase

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Introduction

It was shown by Ehrlich (1885), Vernon (1911*a*, *b*), Battelli and Stern (1912) and others* that indophenol oxidase or indophenolase is one of the most widely distributed oxidizing enzymes found in cells of aerobic organisms.

The presence of this enzyme is usually recognized by the property of cells to oxidize "nadi" reagent (dimethyl-*p*-phenylenediamine + α-naphthol) to indophenol or *p*-phenylenediamine to a di-imine.

Although a great deal of work has been devoted to the study of this enzyme, the literature on the subject still contains a great bulk of inaccurate and controversial statements, and faulty interpretations of observed facts. This was naturally responsible for the introduction of several unsound theories of the mechanism of intracellular respiration.

In this paper we propose to examine the mechanism of the catalytic activity of this enzyme in order to determine to what extent this activity depends on co-operation with cytochrome. This will be followed by a critical examination of some theories dealing with the nature of this oxidase and with the mechanism of cellular oxidations.

Material and methods

Oxidase. The best source of this enzyme is the heart muscle of mammals, and the best method of preparation still remains that of Battelli and Stern (1912). It consists in pounding the heart muscle to a fine pulp, shaking it with 2–3 vol. of slightly alkaline water, centrifuging off the coarse particles, precipitating the supernatant opalescent fluid with very weak acid and resuspending the precipitate in alkaline water. The main deviation from this method, which is now generally adopted, consists in mincing the fresh muscle, washing it thoroughly in water and grinding the washed mince with sand.

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* The extensive literature on this subject has been recently reviewed in a book by Euler (1934) on the chemistry of enzymes.

Throughout this study we have made use of the following procedure, which gave enzyme preparations of approximately constant strength.

Pig's heart muscle was selected because fresh material could be obtained practically every day of the week.

Two hearts cleaned from fat and ligaments are minced, and washed ten times in 5 l. tap water with mechanical stirring, the mince being strained and pressed out each time through muslin. 200 g. of this pulp is mixed with 100 g. fine sand and 250 c.c. M/25 phosphate buffer *p*H 7 and ground for 1½ hr. in a mechanical mortar. The thick paste thus obtained is mixed with 350 c.c. of the same buffer and centrifuged hard. The cloudy fluid is poured off, treated with 100 c.c. M/5 acetate buffer *p*H 4·5, centrifuged, the clear fluid discarded and the solid resuspended in 35 c.c. M/10 phosphate buffer *p*H 7·3. A thick pale brown suspension containing a very strong oxidase is thus obtained.

Cytochrome c was prepared as previously described (Keilin and Hartree 1937). The cytochrome solutions obtained by this method were precipitated by adding 4 vol. of cold acetone, centrifuging, washing the precipitate with acetone and drying in a vacuum desiccator. The red powder so obtained is soluble in water and its properties are in no way modified by the acetone treatment. It has the advantage that it may be preserved indefinitely in an ice-chest. 0·2 c.c. of a 1 % solution was generally added to a manometer flask giving a final concentration of $3{\cdot}5 \times 10^{-5}$ M.

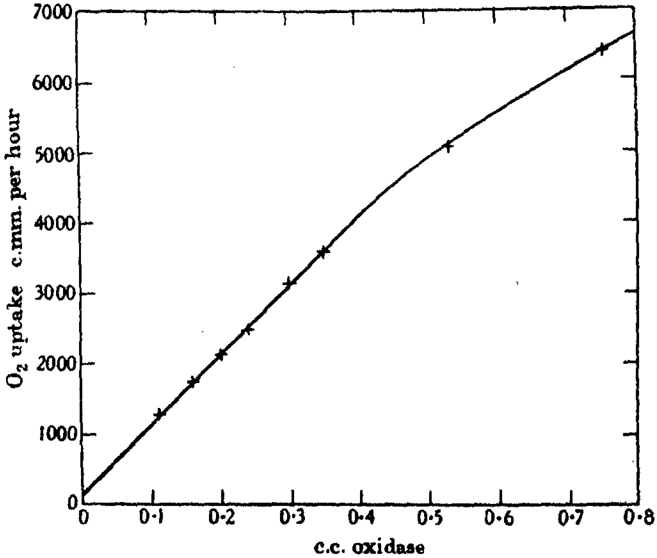

FIG. 1. Oxygen uptake by various amounts of oxidase in presence of M/25 *p*-phenylenediamine. $T = 39°$ C., $pH = 7{\cdot}3$. (Calculated on uptake during first 5 min.) Shaking rate: 180/min.

Preliminary experiments, which are plotted in fig. 1, show that, with rapid shaking, it is possible to obtain an uptake of 4000 cu. mm. O_2/hour without the oxygen diffusion into the liquid becoming a limiting factor.

GENERAL PROPERTIES OF MUSCLE PREPARATION

The preparation thus obtained consists of a very finely divided suspension of muscle tissue. In addition to indophenol oxidase it contains the three components of cytochrome, fumarase and several dehydrogenases, such as succinic, lactic, malic and β-hydroxybutyric. Coenzymes (I and II) are either completely absent or are present in hardly detectable concentrations. The main peculiarity of cytochrome in this preparation is that the concentration of the component *a* is very high, while the concentration of *b* and especially of *c* is very low. However, the complete cytochrome spectrum can be observed with a direct-vision spectroscope in a 2 mm. layer of the oxidase after reduction with $Na_2S_2O_4$. The fresh preparation does not show any oxygen uptake. The cytochrome present is always in the oxidized state and does not undergo reduction even if the preparation is kept in pure nitrogen or if a high concentration of cyanide is added (M/300). This shows that the preparation is completely devoid of metabolites or of substances capable of reducing cytochrome. Reduced cytochrome *c* added to this preparation is rapidly oxidized even in the presence of only small traces of oxygen, which shows the exceptionally high affinity of the preparation for oxygen.

RECONSTRUCTION OF THE OXIDASE-CYTOCHROME SYSTEM

The heart muscle preparation has the property of catalysing the oxidation of many aromatic compounds such as diamines. The chief problem in this connexion is whether these compounds are oxidized directly by the oxidase, in the same manner as are the components of reduced cytochrome, or through the co-operation of cytochrome as in the case of cysteine (Keilin 1930). The most obvious difficulty in solving this problem is the fact that the enzyme preparation still contains the three components of cytochrome, which withstood washing. This difficulty, however, is only apparent. In fact, the preparation already shows an appreciable reduction in the concentration of cytochromes *b* and *c*. Moreover, owing to the destruction of cellular structure and the dispersion of the components of the oxidizing system in a larger volume of fluid, the mutual accessibility of these compounds is greatly decreased. The reduction in the efficiency of such a system, caused mainly by dilution, can be compensated, however, by increasing the concentration of some of its components. This applies not only to washed muscle preparation but also to the preparation obtained from unwashed muscle, thoroughly ground with sand and suspended in buffer solution *p*H 7·3 (see Exp. 7, Table I). These facts, together with spectroscopic observations that all the compounds oxidized by this preparation reduce cytochrome *c*, suggest that the addition of soluble cytochrome *c* to this preparation may increase its catalytic activity towards these compounds. In order to test this possibility, a number of experiments were carried out in Barcroft differential manometers on the rates of oxidation of different compounds catalysed by the enzyme preparation with and without the addition of cytochrome *c*.

The enzyme preparation was diluted with phosphate buffer solution so that only 5 to 10 mg. of dry weight of the preparation was present in 3 c.c. of fluid in the right-hand flask of the manometers.

The substrates were used in as pure a state as possible. Compounds like *p*-aminophenol and *p*-phenylenediamine, which easily undergo autoxidation, had to be recrystallized. The substrates were always added by means of dangling tubes which were dislodged only after the temperature of the manometers had been equilibrated. All experiments were carried out at 39° C. in M/10 phosphate buffer, *p*H 7·3.

The results of these experiments, which are shown in Tables I, II and III and figs. 2 and 3, can be summarized as follows:

(1) The addition of 10^{-5}–10^{-4} M cytochrome *c* to heart muscle preparation greatly increases the rate of catalytic oxidation of different diamines and polyphenols by this preparation. While the rate of oxidation of *p*-phenylenediamine is increased up to seven times, that of other compounds (e.g. hydroquinone) may be increased 30–40 times. The rate of oxidation of hydroquinone by 0·03 c.c. oxidase is doubled on addition of 4×10^{-7} M cytochrome *c*.

(2) A good preparation of enzyme, without the addition of cytochrome *c*, catalyses the oxidation of *p*-phenylenediamine much better than that of other substrates (such as *o*-phenylenediamine, *p*-aminophenol, hydroquinone, adrenaline and catechol).

(3) The much slower catalytic oxidation of all these compounds without addition of cytochrome *c* is certainly due to cytochrome already present in the oxidase preparation. This cytochrome seems to be more effective for the oxidation of *p*-phenylenediamine than for the oxidation of other compounds.

(4) Substances like hydroquinone and adrenaline,* which are hardly oxidized by the enzyme preparation alone, may be oxidized by the enzyme

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* Our results with the oxidation of adrenaline have been confirmed by Green and Richter (1937) who have extended these experiments to analogous compounds.

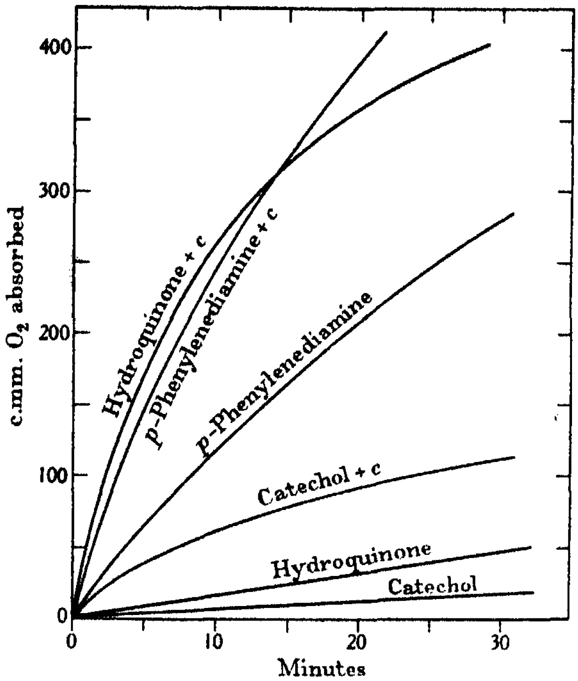

FIG. 2. Oxygen uptake by 0·2 c.c. oxidase in presence of M/50 substrates. 0·2 c.c. 1 % cytochrome *c*. $T = 39°$ C.; *p*H = 7·3.

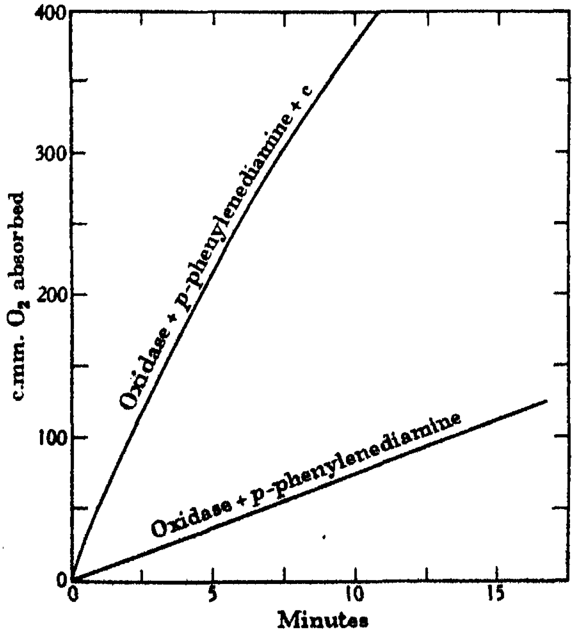

FIG. 3. Oxygen uptake by oxidase (0·2 c.c.) after freezing three times in solid CO_2 and acetone. M/50 *p*-phenylenediamine; 0·2 c.c. cytochrome *c*. $T = 39°$ C.; *p*H = 7·3.

on addition of cytochrome *c*, as fast as, or even faster than, *p*-phenylenediamine.

(5) All these experiments clearly demonstrate that the mechanism of the catalytic oxidation of these diamines and polyphenols is similar to that of cysteine (Keilin 1930). In other words, the oxidation of these compounds is not catalysed directly by the oxidase but through the co-operation of cytochrome.

(6) The only catalytic property which can be definitely ascribed to this oxidase is the oxidation of reduced cytochrome. Cytochrome is therefore the only natural and the only known compound which is catalytically oxidized by this enzyme. Following the general rule of nomenclature adopted for other enzymes, the correct name of this oxidase is, therefore, *Cytochrome oxidase*.

These experiments finally explain some of the marked discrepancies in the observations of previous workers on the capacity of muscle preparations to oxidize different diamines and polyphenols and on the relative rates of such oxidation reactions. It is obvious now that the rate of oxidation of such compounds by a muscle preparation will depend on the degree of washing, pounding and dilution, and the cytochrome content of the preparation.*

In presence of *p*-phenylenediamine the preparation used for the experiments of Table II shows a considerable oxygen uptake without addition of cytochrome *c*. This property enables the difference in behaviour of the enzyme towards *p*-phenylenediamine and other substrates to be clearly demonstrated.

EXTRACTION OF OXIDASE FROM UNGROUND MUSCLE

Minced pig's heart was washed 5 times with distilled water and once with 0·5 % NaCl. 45 g. was allowed to stand with 100 c.c. M/10 Na_2HPO_4 for 48 hours at 5° C. and the fluid *Y* then sucked off. It contained a little haemoglobin and was slightly opalescent. When passed through a coarse Seitz filter (K-Schichte) a practically clear solution Y_1 was obtained, and by passing the fluid four times through the same filter it was completely clarified

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* That our previous results (Keilin 1929) did not agree with those of Szent-Györgyi (1925) has recently been pointed out (Euler, 1934, p. 412). Unfortunately, the numerical data given by Szent-Györgyi in his paper in a table on p. 78 and in fig. 2, owing to some mistake in calculation, are not correct. Moreover, as he used about 2 g. of muscle preparation per manometric flask, the diffusion of oxygen into this thick suspension was probably the limiting factor in the rate of oxygen uptake in his experiments.

TABLE I. OXIDATION OF DIFFERENT SUBSTANCES BY OXIDASE PREPARATION WITH (+) AND WITHOUT (−) ADDITION OF SOLUBLE CYTOCHROME *C*

Oxidase preparation, 0·2–0·3 c.c. of stock solution diluted three times which corresponds to 5–7·5 mg. of dry weight; cytochrome c, 0·2 c.c. of 1 % solution; substrate, M/50; *p*H 7·3; *T* = 39°; shaking 160–200 times per minute.

Exp. no.	Oxidase	Cytochrome *c*	*p*-Phenylene-diamine	Time (min.)	O_2 uptake cu. mm.
1	+	−	+	25	185
	+	+	+	25	510
2	+	−	+	25	50
	+	+	+	25	350
3	+	−	+	15	98
	+	+	+	15	254
4	+	−	+	10	70
	+	+	+	10	375
5	+	−	+	15	160
	+	+	+	15	425
6	+	−	+	10	40
	+	+	+	10	265
7	+	−	+	15	165
	+	+	+	15	400
8	+	−	Hydroquinone	15	10
	+	+	+	15	320
9	+	−	+	15	15
	+	+	+	15	335
10	+	−	+	15	5
	+	+	+	15	180
11	+	−	Catechol	15	1
	+	+	+	15	54
12	+	−	+	20	8
	+	+	+	20	90
13	+	−	*o*-Phenylenediamine	15	3
	+	+	+	15	49
14	+	−	Adrenaline	15	13
	+	+	+	15	394
15	+	−	*p*-Aminophenol	15	11
	+	+	+	15	129

Exp. 4. After freezing the oxidase three times.

Exps. 5, 9. Oxidase prepared from skeletal muscle (sheep) and containing very little cytochrome.

Exp. 7. Unwashed heart muscle ground with sand.

TABLE II. Q_{O_2} OF OXIDATION OF DIFFERENT SUBSTRATES (M/50) BY THE SAME HEART MUSCLE PREPARATION WITH (+*c*) AND WITHOUT (−*c*) THE ADDITION OF CYTOCHROME *c* ($3{\cdot}5 \times 10^{-5}$ M)

$T = 39°$ C.; *p*H 7·3.

Substrate	−*c*	+*c*
p-Phenylenediamine	70	181
o-Phenylenediamine	2	35
p-Aminophenol	8	102
Hydroquinone	4	128
Adrenaline	9	280
Catechol	1	38

TABLE III. OXIDATION OF *p*-PHENYLENEDIAMINE (M/50) BY VARYING AMOUNTS OF OXIDASE, WITH (+*c*) AND WITHOUT (−*c*) ADDED CYTOCHROME *c* (0·2 c.c. 1 % SOLUTION)

$T = 39°$ C.; *p*H 7·3.

Vol. of oxidase (c.c.)	O_2 (cu. mm.) uptake in 15 min.		Ratio of velocities
	−*c*	+*c*	
0·1	200	387	1·94
0·05	95	277	2·92
0·025	50	172	3·44
0·01	21	79	3·76

TABLE IV. 1 C.C. OXIDASE; M/50 *p*-PHENYLENEDIAMINE; 0·2 C.C. 1 % CYTOCHROME *c*

$T = 39°$ C.; *p*H = 7·3.

Y	... ...	82 cu. mm. O_2 in 10 min.
Y_1	... ...	23 ,, ,,
Y_2	... ...	9 ,, ,,

(Y_2). The effect of these filtrations on the ability to oxidize *p*-phenylenediamine is shown in Table IV.

These experiments provide further proof of the insolubility of cytochrome oxidase in heart muscle preparations (see p. 184).

EFFECT OF CARBON MONOXIDE ON CYTOCHROME OXIDASE

Previous results (Keilin 1930) supported the view that the oxidase is inhibited by CO in the dark. The experimental results given in Table V show, however, that a marked inhibition is not obtained unless the other components of the system, i.e. cytochrome *c* (see also Table III) and substrate, are present in excess. When this is not the case the oxidase does not work at full capacity, consequently part of it may be poisoned without affecting the oxidative activity of the system as a whole.

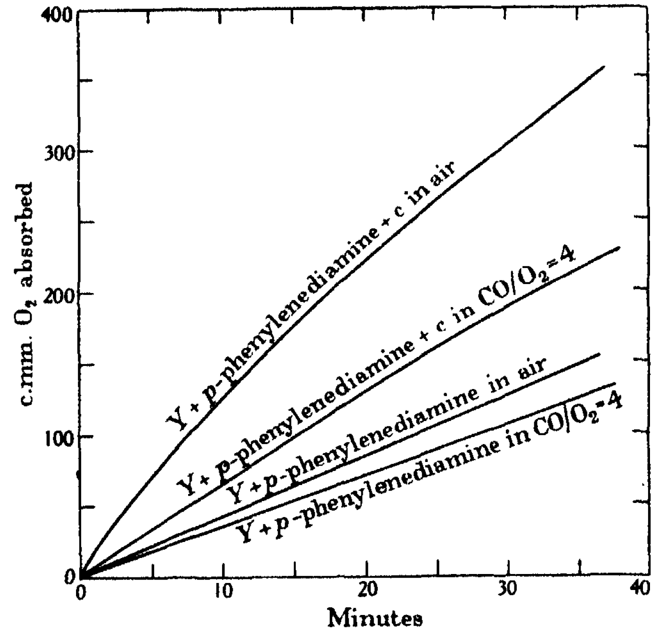


FIG. 4. Effect of CO on the oxygen uptake of oxidase *Y* (1 c.c.) in presence of M/25 *p*-phenylenediamine, with and without cytochrome *c*.

TABLE V. OXIDASE DILUTED TEN TIMES BEFORE USE

$T = 39°$ C.; *p*H 7·3.

			O_2 (cu. mm.) uptake in 15 min.			$K = \frac{n}{1-n}\frac{[CO]}{[O_2]}$
			A	A_1		
Oxidase (c.c.)	*p*-Phenylene-diamine	*c* (c.c.)	In air	In $\frac{CO}{O_2} = 4$	% inhib.	$\left[n = \frac{A_1}{A}\right]$
0·4	M/25	—	113	116	—	—
0·4	M/25	0·2	217	190	12	28
0·2	M/25	—	60	59	—	—
0·2	M/25	0·2	132	98	26	11·5

The fluid *Y* (described above) gave similar results when tested with CO (Table VI, fig. 4).

It is a well-known fact that the enzymic oxidation of *p*-phenylenediamine is accompanied by considerable polymerization and colour formation. To what extent the enzyme system catalyses each of these many transformations it is impossible to say. Therefore in order to obtain more reliable evidence for the effect of CO on cytochrome oxidase, it is necessary to use as substrate

TABLE VI. EFFECT OF CO ON THE OXIDATION OF *p*-PHENYLENEDIAMINE BY THE PREPARATION *Y* WITH $(+c)$ AND WITHOUT $(-c)$ ADDITION OF 0·2 C.C. CYTOCHROME *c*

1 c.c. oxidase Y + M/25 *p*-phenylenediamine	O_2 (cu. mm.) uptake in 30 min. In air	O_2 (cu. mm.) uptake in 30 min. In $\frac{CO}{O_2} = 4$	% inhib.	$K = \frac{n}{1-n} \frac{[CO]}{[O_2]}$
$-c$	127	107	16	21
$+c$	300	180	40	6

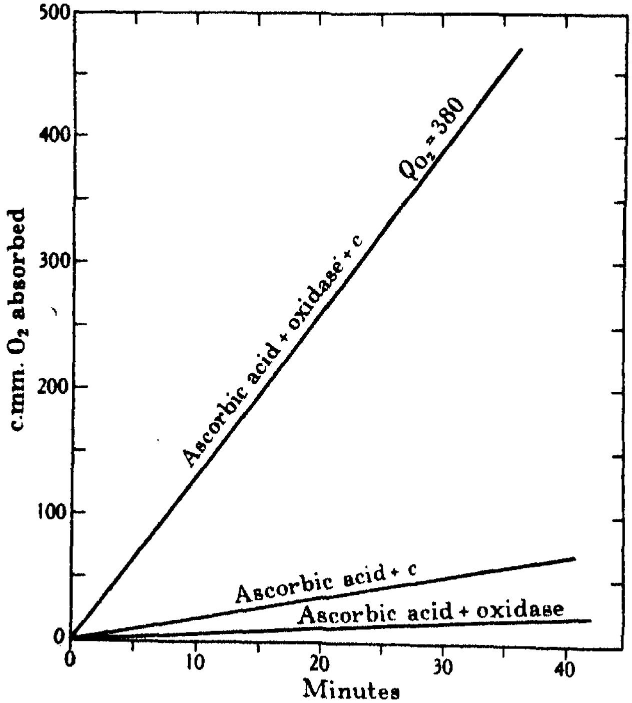


FIG. 5. Oxygen uptake by oxidase (0·05 c.c.) with ascorbic acid (20 mg.) and cytochrome *c* (0·3 c.c.).

a material which, while reducing cytochrome, is oxidized to a stable substance which can undergo no further changes and which is without effect on the oxidase. Under these circumstances the oxygen uptake would be constant over a long period. A substance fulfilling these conditions is ascorbic acid, which is rapidly oxidized by the system (fig. 5) and the oxidation of which is strongly inhibited by CO, the partition constant *K* being 2·2 (fig. 6).

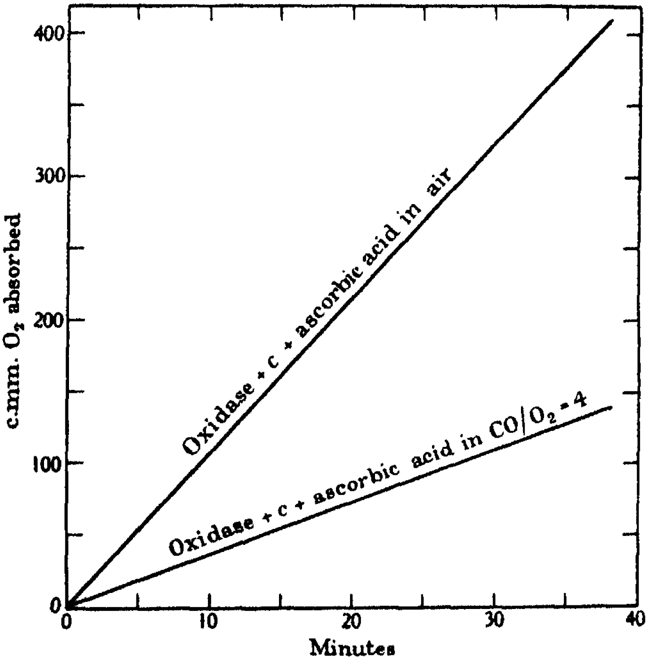


FIG. 6. Effect of CO on oxidase (0·03 c.c.) + ascorbic acid (20 mg.) + cytochrome *c* (0·3 c.c.).

SOME PREVIOUS MISLEADING STATEMENTS CONCERNING THE NATURE AND OCCURRENCE OF CYTOCHROME OXIDASE

The crude colour tests with *p*-phenylenediamine, "nadi" or other reagents, which were not checked manometrically, were to a great extent responsible for many incorrect statements and wrong interpretations of the nature and properties of cytochrome oxidase.

Thus, Remesow's supposition (1932) as to the cholesterine nature of this enzyme was solely based on some colour reactions given by cholesterine solutions or emulsions; reactions which are not accompanied by a measurable oxygen uptake.

Again, Bigwood and Thomas's statement (1935) as to the presence of indophenol oxidase in milk was also based on a colour test. That this statement is incorrect is obvious from the facts (1) that manometric experiments do not reveal any appreciable oxygen uptake taking place during this reaction, and (2) that reduced cytochrome *c* is not oxidized by milk.

On the other hand, according to Harrison (1929), it is hardly necessary even to postulate the existence of an enzyme such as indophenol oxidase. The oxidation of *p*-phenylenediamine in muscle preparation he explains as a secondary or coupled oxidation by means of a peroxidase or a haematin compound acting in conjunction with H_2O_2 formed in a primary oxidation of substances such as glutathione or hypoxanthine.

That this view cannot be accepted is clearly shown by the following considerations (see also Keilin and Hartree 1936):

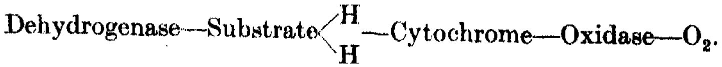
(1) No H_2O_2 can be detected in a strong enzyme preparation even on saturating it with oxygen.

(2) The formation of H_2O_2 in an enzyme preparation must be accompanied by a corresponding oxygen uptake, whilst in a strong oxidase preparation no oxygen uptake can be detected.

(3) The oxygen uptake of the secondary oxidation is usually equal to or slightly lower than that of the primary reaction, but while the Q_{O_2} of our enzyme preparation, on addition of substrates, may be as high as 300, it is zero in their absence.

OXIDASE-CYTOCHROME SYSTEM. GENERAL PROPERTIES

The study of cellular respiration strongly supports the view that one of the most widely distributed respiratory systems of the cell can be schematically represented as follows:



$$\text{Dehydrogenase—Substrate}\begin{smallmatrix}\diagup \mathrm{H}\\ \diagdown \mathrm{H}\end{smallmatrix}\text{—Cytochrome—Oxidase—}O_2.$$

In this system cytochrome is reduced by the metabolites activated by their specific dehydrogenases and reoxidized by cytochrome oxidase and oxygen (Keilin 1925, 1929).

The more recent work on this subject suggests that only certain activated metabolites can reduce the oxidized cytochrome directly while others do it through the medium of additional carriers or in co-operation with other oxido-reduction systems.*

The activity of cytochrome in this system is accompanied by a valency change of the iron of its three components. It should also be borne in mind that while the reduction of cytochrome is inhibited by urethane and substances similar to the substrate which can act as competitive inhibitors, the reoxidation of cytochrome is inhibited by KCN, H_2S, NaN_3 and CO.

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* This problem will be discussed in a separate paper.

VIEWS OF SHIBATA AND HIS CO-WORKERS

A new interpretation of the mechanism of intracellular respiration was proposed recently by Shibata (1935), Tamiya and their co-workers, and a large amount of work has been published by the Japanese school in support of their views. For reasons which will be explained later they have found it necessary to ascribe to one of the components of the system the property of reversible oxygenation. At first, they considered that cytochrome is this component which, like haemoglobin, undergoes oxygenation and deoxygenation without changing the valency of its iron. The molecular oxygen given off by cytochrome is utilized, according to them, by the indophenol oxidase which is then reduced directly by the dehydrogenase system.

They have ultimately modified this view by restoring cytochrome to its original place, and introducing an "oxygen transporting enzyme", transferring molecular oxygen to the indophenol oxidase and thus forming a respiratory system which can be represented as follows:

$$O_2\text{—}O_2\ \overset{\overset{\text{CO}}{\downarrow}}{\text{transporting}}\text{ enzyme—Indoph. }\overset{\overset{\text{KCN}}{\downarrow}}{\text{oxidase}}\text{—Cytochrome—dehydrogenase system.}$$

According to these workers, indophenol oxidase, when free from other components of the system, although still strongly inhibited by KCN is no longer affected by CO. They believe this can be demonstrated by the study of a clear solution of oxidase which they have obtained from yeast cells and heart muscle and by the study of an oxidase extracted from a mushroom, *Lactarius*. On the other hand, the effect of CO on respiration they ascribe to its combination with another component of the system. This component, they believe now, to be the oxygen transporting enzyme which, like haemoglobin, combines reversibly with molecular oxygen, undergoing oxygenation which is not accompanied by a change of valency of its iron. CO and KCN therefore inhibit the respiration by acting on two different components.

We shall now examine separately the three main points of their work, namely: (1) the properties of their soluble indophenol oxidase extracted from yeast and heart muscle, (2) the relationship between the oxidase of *Lactarius* and the indophenol oxidase of yeast cells or heart muscle, and (3) the existence of an additional component undergoing oxygenation.

(1) It was claimed recently by Yamagutchi, Tamiya and Ogura (1936) that they have obtained from yeast cells and heart muscle a clear solution of an indophenol oxidase which is sensitive to KCN but not to CO.

The preparation of their enzyme from heart muscle up to the precipitation of the opalescent fluid does not differ essentially from the preparation described in this paper. The precipitate is however extracted with

M.K_2HPO_4, the suspension centrifuged, the opalescent fluid (fraction *A*) collected, filtered through a Seitz filter to give a clear solution, neutralized with KH_2PO_4, and used as enzyme preparation (fraction *B*). This fluid gives, according to them, a very strong colour reaction with "nadi" reagent, and this reaction is inhibited by KCN but not by CO. The only few manometric data concerning this enzyme of heart muscle are found in a paper by Yamagutchi (1937, p. 189) on *Bacillus pyocyaneus* and taken from a paper by Yamagutchi and Ogura published in Japanese.

We have repeated the experiments of these workers but could not confirm their results. While 1 c.c. of fluid *A* (after dialysis) takes up with *p*-phenylenediamine about 330 cu. mm. of oxygen in 20 min., the clear fluid *B* obtained after filtration through the coarsest Seitz filter does not take up any measurable amount of oxygen under the same conditions, and does not oxidize reduced cytochrome *c*. The preparation obtained by this method does not, therefore, contain indophenol or cytochrome oxidase.

If, on the other hand, the preparation of Yamagutchi and Ogura did produce an appreciable oxygen uptake with *p*-phenylenediamine it can only be explained by an incomplete filtration (see p. 178) or by the presence of metallic impurities (e.g. 0·1 mg. copper as sulphate will cause an oxygen uptake of 560 cu. mm./hr. with M/50 *p*-phenylenediamine in a Barcroft manometer). The presence of enzyme in such a preparation should be detectable by a very slight opalescence. The fact that such an enzyme preparation is not poisoned by CO is probably due to a deficiency in cytochrome which could be easily compensated by the addition of cytochrome *c* (cf. p. 179).

(2) The oxidase of *Lactarius*, like that of many other wild mushrooms, oxidizes a large variety of substances, such as catechol, pyrogallol, hydroquinone, *p*-cresol, *p*-phenylenediamine, *p*-aminophenol, etc. It was shown previously (Keilin 1929) that the polyphenol oxidase of potatoes which oxidizes mainly orthodihydroxyphenols is very strongly inhibited by CO. This inhibition, which is perfectly reversible, is not affected by light. According to Shibata and his co-workers, the oxidation of *p*-phenylenediamine by *Lactarius* oxidase is not inhibited by CO. On the other hand, we have found that while the oxidation of *p*-phenylenediamine by the oxidase from *Lactarius* and some other wild mushrooms is not affected by CO, the oxidation of *p*-cresol and catechol by the same enzyme is distinctly inhibited by CO.* The oxidase of *Lactarius* belongs therefore to the same

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* It is surprising that instead of testing the effect of CO on the oxidation of *p*-cresol and catechol by *Lactarius* oxidase Yakushiji (1937) endeavours to find a polyphenol oxidase in an entirely different mushroom, *Octaviania columellifera*, which is inhibited by CO.

group of enzymes as the polyphenol oxidase of higher plants. It differs from the indophenol or cytochrome oxidase of some bacteria, yeast and animals in many important characteristics which can be briefly summarized as follows:

Indophenol or Cytochrome oxidase is either not soluble or is intimately attached to the insoluble material of the cells. It is rapidly destroyed by drying, and by treatment with alcohol or acetone. It oxidises cytochrome very rapidly. Its catalytic activity is intimately bound with cytochrome and it oxidizes various diamines and polyphenols only through cytochrome.

Oxidase from Lactarius is soluble, is resistant to drying and to treatment with alcohol, acetone and salts (which are used for the purification of this enzyme). It oxidizes cytochrome *c* slowly and does not require its co-operation for the oxidation of the great variety of phenols and amines. It reacts with these substances either directly or, in some cases, possibly through the co-operation of a quinone.

Polyphenol oxidase from higher plants and cultivated mushrooms differs from the *Lactarius* oxidase in oxidizing only the orthodihydroxyphenols.

All these oxidases have several properties in common: they show a high affinity for oxygen and are inhibited by KCN, H_2S, NaN_3 and CO.

(3) Having arrived at the conclusion that CO does not inhibit the oxidase-cytochrome system, Shibata and his co-workers were compelled to find a component of the system which reacts with CO. In their recent papers they ascribed the effect of CO to the "oxygen transporting enzyme" which like haemoglobin combines reversibly with molecular oxygen and CO. It is, however, difficult to understand why these authors have to introduce into the system a component undergoing "oxygenation", as substances like haemochromogens, as is well known, combine reversibly with CO and their catalytic activity depends on the valency changes of iron. That the introduction of their "oxygenating" component is completely unnecessary becomes even more obvious when in some of their systems they deprive it of the property of reacting with carbon monoxide (Yamagutchi 1937, pp. 193–8).

We can say in conclusion that the results of our study strongly oppose the views on the mechanism of intracellular respiration developed by Shibata and his co-workers.

SUMMARY AND CONCLUSIONS

1. The addition of 10^{-5} to 10^{-4} M cytochrome *c* to heart muscle oxidase preparation greatly increases the rate of catalytic oxidation of a number of diamines and polyphenols such as *p*-phenylenediamine, *p*-aminophenol, hydroquinone, adrenaline, catechol, etc.

2. All these compounds readily reduce a solution of oxidized cytochrome *c*.

3. The rate of oxidation of these compounds by oxidase preparation may increase, on addition of cytochrome *c*, up to 30–40 times.

4. The much slower oxidation of these compounds without addition of cytochrome *c* is due to cytochrome already present in the oxidase preparation.

5. The oxidation of all these compounds therefore is not catalysed directly by indophenol oxidase but through co-operation with cytochrome. In other words, the indophenol reaction of cells and their extracts takes place through cytochrome.

6. The only catalytic property which can be definitely ascribed to the muscle oxidase (known as indophenol oxidase) is the oxidation of reduced cytochrome. The correct name for this enzyme, should, therefore, be *cytochrome oxidase*.

7. The explanations as to the nature, distribution and catalytic activity of this enzyme proposed by Remesow, Bigwood, Harrison, and Shibata with his co-workers, are discussed and shown to be incorrect.

8. There is no necessity to assume the existence in the respiratory system of the cell of a component undergoing reversible "oxygenation" as was proposed by Shibata and his co-workers.

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Polyphenol oxidase
Purification, nature and properties

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1. Introduction

In this paper we propose to describe a comparatively simple method of preparation and purification of a polyphenol oxidase. The study of this highly purified enzyme makes possible the determination of its active group and of its main properties which are masked in crude preparations even when they are highly active.

Material. The best source of this enzyme is presented by cultivated mushrooms which belong to one of the variety of *Agaricus* or *Psalliota campestris* somewhat modified by continual and intense cultivation. This material has the advantage of being very rich in enzyme and of being available in large quantities practically all the year round.

General course of preparation

The mushrooms are minced and extracted with water. The extract is precipitated with ammonium sulphate, the precipitate is suspended in water and dialysed until free from ammonium sulphate. Further steps of concentration and purification of the enzyme consist in fractional precipitation with lead acetate, adsorption on tricalcium phosphate gel followed by a fractional precipitation with acetone in presence of lead acetate, a second adsorption on tricalcium phosphate and a final fractional precipitation with acetone. By this method a highly purified enzyme preparation can be easily obtained within 10 days using only a few reagents, i.e. ammonium sulphate, lead acetate, tricalcium phosphate gel, alkaline phosphate and acetone.

2. Estimation of activity and concentration of enzyme

The activity of polyphenol oxidase in a preparation was estimated by two different methods: (1) *colorimetric*, based on the oxidation of pyrogallol to purpurogalline and expressed in terms of purpurogalline number (P.N.), and (2) *manometric*, based on the oxidation of pyrocatechol and expressed in terms of Q_{O_2}.

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* Beit Memorial Research Fellow.

(1) *The colorimetric method* is very similar to the standard method introduced by Willstätter for estimation of peroxidase activity.

A definite amount of enzyme preparation is added to 250 c.c. water containing 1 g. of resublimed pyrogallol, and the mixture is shaken in air at room temperature (20° C.). After 5 min. shaking, the oxidation of pyrogallol is completely stopped by addition of 5 c.c. 20 % H_2SO_4. The purpurogalline is extracted with pure redistilled ether and estimated colorimetrically by comparison with a standard solution of purpurogalline. The concentration of enzyme used for an estimation was so adjusted that the amount of purpurogalline formed was not less than 1 mg. and not more than 4 mg. The activity of enzyme is expressed as purpurogalline number or P.N. which is analogous to Willstätter's P.Z. and represents the mg. of purpurogalline formed from pyrogallol in 5 min. at 20° C. per 1 mg. of dry weight of enzyme preparation. The amount of enzyme present in a preparation is expressed in terms of enzyme units (E.U.). One unit corresponds to the amount of enzyme which produces 1000 mg. purpurogalline in 5 min. at 20° C.

(2) *Manometric determination* of oxidase activity is carried out in the usual way in Barcroft differential manometers at 20° C. The right-hand flask receives 5 or 10 mg. pyrocatechol dissolved in 3 c.c. 0·03 M phosphate solution of *p*H 7·3 and a dangling tube containing 0·3 c.c. of enzyme. The left-hand flask receives only 3·3 c.c. water. When the temperature of the flasks is equilibrated the taps are closed, the dangling tubes are dislodged and the manometers are shaken about 180 times/min. The manometers are read at the end of the first and second minutes and then every 2 or 4 min.

The activity of the enzyme is expressed as Q_{O_2}—or oxygen uptake by pyrocatechol catalysed by 1 mg. of dry weight of the oxidase preparation in 1 hr. at 20° C. calculated from the velocity during the first 2 min of the reaction.

3. METHOD OF PREPARATION AND PURIFICATION OF POLYPHENOL OXIDASE

(1) *Extraction of enzyme from mushrooms*

15 kg. of fresh, young cultivated mushrooms are cut in slices and minced finely with a geared mincer. The pulp is mixed with about 1 kg. of fine

sand and pressed out through linen at first by hand and then by means of a hydraulic press, giving 5 l. extract. The residue is mixed with water and pressed out, giving 2·5 l., and the almost dry residue is mixed with about 3 l. water, 1 kg. of sand and ground in four separate lots in a mechanical porcelain mortar, which takes about 3–4 hr. The pulp is pressed out giving 2·5 l. fluid, the extraction of the pulp with water being repeated twice. All the extracts collected give 15 l. of brown turbid fluid (fraction *A*), containing 48 mg. dry weight per c.c. The enzyme activity of this preparation or P.N. is 0·45; in other preparations it varied from 0·25 to 0·65. The enzyme content or E.U. is about 320; in other preparations it varied from 150 to 450. The Q_{O_2}, estimated by oxidation of catechol, is 785; in other preparations it varied from 600 to 1200.

The success of the extraction depends on very fine mincing, grinding the mince to a fine pulp and a thorough pressing. However, a fairly good extraction giving naturally a smaller yield can be obtained by using an ordinary large mincer and a hand botanical press, as used for extraction of fruit juice, without previously grinding the pulp in a mechanical mortar.

The extract *A*, when left overnight, often shows an increase in enzyme content which may be due either to the removal of some substances inhibiting the reaction or to the liberation of an active enzyme from an inactive complex which it may form with some other substances present.

(2) *Precipitation with ammonium sulphate and dialysis*

The extract *A*, which is left overnight, is brought to *p*H 4·8–5 with acetic acid and saturated with ammonium sulphate by mixing it thoroughly with about 11 kg. of this salt. In about 2 hr. the precipitate aggregates and has a tendency to rise. The mixture is poured on to several large Büchner funnels covered with two layers of filter paper and the fluid is sucked off by a vacuum pump leaving a very dark, almost black, thick mass of precipitate. Using only two funnels 27 cm. in diameter, the filtration is complete in a few hours. The dark precipitate is collected, suspended in 1·5 l. water and dialysed in two large cellophane sacs against running tap water. 8–12 hr. later, when most of the ammonium sulphate has been removed, the fluid is transferred into long cellophane tubes (2 cm. in diameter) and dialysed against running tap water for 10–20 hr. to complete the dialysis. 4 l. of dark brown, turbid fluid *B* from the cellophane tubes, when examined for enzyme activity, gives the following results: Dry weight = 24 mg. per c.c.; P.N. = 3·3; E.U. = 320; Q_{O_2} = 5500.

(3) *Purification with lead acetate*

Fluid *B* is diluted with cold distilled water to 8 l. and treated with a very small amount of lead acetate just sufficient to remove the turbidity and most of the colour.

The amount of lead acetate required for this purpose can easily be determined using four 20 c.c. samples of this fluid treated with different amounts of lead acetate. Saturated lead acetate solution is diluted 10 times with CO_2-free distilled water and the following volumes added to the four samples of fluid: 0·25, 0·35, 0·5 and 0·75 c.c. Immediately after the addition, the precipitate in each sample is centrifuged off. The fluid of the first sample remains dark and turbid, while that of the second sample is brownish, almost clear and contains 80 % of the enzyme. The fluid of the third sample is completely clear, only slightly brownish and contains 60 % of enzyme. The fourth sample is also clear, almost colourless, but contains only 35 % of the enzyme. This preliminary test shows that 0·5 c.c. of dilute lead acetate per 20 c.c. of fluid *B* is the most suitable concentration for our purpose.

Accordingly, the fluid *B* was treated in ten lots of 800 c.c. each receiving 20 c.c. of lead acetate (1 part saturated solution + 9 parts CO_2-free distilled water) and the precipitate immediately centrifuged off. 7800 c.c. of clear slightly brown fluid C_1 collected in this way has dry weight of 1·6 mg./c.c.; P.N. = 14 and E.U. = 175.

(4) *First fractional adsorption on tricalcium phosphate*

The fluid C_1 is mixed with 2 g. of tricalcium phosphate gel, adjusted to *p*H 7·0–7·2 and left overnight.

The next morning the fluid is filtered off through a fluted Chardin filter paper, leaving behind a black residue. The clear, slightly yellow fluid which still contains 150 E.U., is acidified to *p*H 6·6–6·7 and mixed with 9 g. of freshly prepared gelatinous tricalcium phosphate. After standing 30 min. the mixture is centrifuged and the fluid which contains not more than 6 E.U. is discarded. The solid is extracted three times with 0·5 M K_2HPO_4; the mixtures being left standing for ½, 2 and 4 hr. respectively before being centrifuged.

The phosphate elutions are united and dialysed overnight against distilled water.

(5) *Second fractional adsorption on tricalcium phosphate*

800 c.c. of brownish fluid thus obtained is acidified to *p*H 6·7 and treated at first with only 2 g. calcium phosphate gel which, after a thorough mixing,

is centrifuged off giving the cake C_2. The fluid is now mixed with 4 g. of the gel which, on centrifuging, gives the second cake D. The two cakes are eluted separately with alkaline phosphate and the elutions dialysed for 24 hr. against distilled water giving two enzyme fractions C_2 and D.

The fraction C_2 consists of 360 c.c. of clear, slightly brown fluid. Its dry weight is 1 mg./c.c., P.N. = 75, $Q_{O_2} = 112{,}000$, and it contains 27 E.U.

The fraction D consists of 300 c.c. of clear, slightly yellow fluid with dry weight of 0·8 mg./c.c., P.N. = 310, $Q_{O_2} = 462{,}000$ and it contains 75 E.U.

(6) *First fractional precipitation with acetone in presence of lead acetate*

The enzyme solution D only was used for further purification. 300 c.c. of this solution is concentrated and washed by ultra-filtration on Bechhold's graded ultra filter membranes. The enzyme solution is poured on to a "4½ %" membrane, the surface of which is constantly swept by a fine brush driven by a small electric motor. The enzyme is retained on this membrane, and it can be rapidly concentrated and washed with water, the latter being sucked off by a vacuum pump.

The solution D, reduced thus to 60 c.c. of slightly cloudy fluid, is treated with 1·2 c.c. of 1/10 saturated solution of lead acetate and 3 c.c. of cold acetone (= 5 % acetone).

The dark mucilaginous precipitate is centrifuged off and the supernatant fluid is treated with 40 c.c. acetone, bringing its final concentration to about 40 %. The fluid is adjusted with acetate buffer to *p*H 5·5 and, after 5 min. standing, is centrifuged. The precipitate D_1 is dissolved in 35 c.c. of 5×10^{-4} phosphate buffer *p*H 7·3. The supernatant fluid mixed with 2 volumes of acetone gives a second precipitate D_2 which is dissolved in 30 c.c. of the same phosphate buffer.

Solutions D_1 and D_2 were estimated after dialysis overnight against glass distilled water, and gave the following results: Solution D_1 = 30 c.c. (almost colourless), dry weight = 3·3 mg./c.c., P.N. = 380, $Q_{O_2} = 560{,}000$, E.U. = 37. Solution D_2 = 35 c.c., dry weight = 1·1 mg./c.c., P.N. = 220, E.U. = 9.

(7) *Second precipitation with lead acetate*

Solution D_1 (30 c.c. containing 37 E.U.) is treated with 2·5 c.c. of 1/100 saturated solution of lead acetate. The precipitate is centrifuged off and the fluid dialysed for 24 hr. against glass distilled water. The slight precipitate formed during dialysis is centrifuged off and the fluid D_3 tested for enzyme, giving the following results: P.N. = 550, $Q_{O_2} = 800{,}000$, E.U. = 34.

(8) *Third absorption on tricalcium phosphate*

Solution D_3 is mixed with 100 mg. calcium phosphate gel and centrifuged. The supernatant fluid is diluted with cold glass distilled water to 200 c.c., treated with 400 mg. calcium phosphate gel, and centrifuged. The cake is carefully eluted with 0·05 M K_2HPO_4 and the elution dialysed against glass distilled water, gives 21 c.c. of colourless solution *E* containing 1·5 mg. dry weight/c.c.; P.N. = 670, E.U. = 21.

(9) *Second fractional precipitation with acetone in presence of lead acetate*

Solution *E* is cooled and treated with 1·2 c.c. 1/100 saturated solution of lead acetate and 1·3 c.c. cold acetone which brings the concentration of the latter to about 5%. The precipitate so formed is centrifuged off. The supernatant fluid is fractionated by two successive precipitations with acetone, the first with 4·8 c.c. (or 22%), the second with 9 c.c. (or 41%). The two precipitates are each dissolved in 8 c.c. of 5×10^{-4} M phosphate *p*H 7·3 and dialysed against glass distilled water, giving two colourless solutions E_1 and E_2. Examination of these solutions gives the following results: E_1 = 7·1 c.c., dry weight = 1·1 mg./c.c., P.N. = 640, Q_{O_2} = 870,000, E.U. = 5. E_2 = 6 c.c., dry weight = 1·6 mg./c.c., P.N. = 940. Q_{O_2} = 1,170,000, E.U. = 9.

The fraction E_2, still containing 9·6 mg. dry weight of enzyme, the activity or P.N. of which is 940, is the purest preparation of polyphenol oxidase so far obtained.

The oxidase activity of this preparation is between 6000 and 7000 times greater than the oxidase activity of mushrooms (P.N. = 0·14) and about 2100 times greater than the activity of the first crude extract (P.N. = 0·45).

4. NATURE OF POLYPHENOL OXIDASE

(1) *Previous work and preliminary investigation*

That polyphenol oxidase is very sensitive to KCN poisoning has already been shown by Kastle and Loevenhart (1901) and others. More recently, it was shown (Keilin 1929, 1936) that polyphenol oxidase is also inhibited by H_2S, NaN_3 and by CO. These observations, taken together with the facts that the enzyme is thermolabile and undergoes destruction when treated with weak acids and alkalies, suggest that the enzyme is a protein combined with a metal such as iron, manganese or copper. That the active group of this enzyme contains neither iron nor manganese is already shown by the preparations of P.N. about 300. In such preparations no trace of haematin can be detected even in presence of pyridine and $Na_2S_2O_4$. Moreover, such preparations contain only 0·007 % Fe and less than 0·003 % Mn, which suggests that both these metals are present only as absorbed impurities.

It was found recently by Kubowitz (1937) in the course of purification of the polyphenol oxidase of potatoes that there is a proportionality between the activity of different enzyme fractions and their copper content. The activity of the enzyme preparations he estimated by the rate of oxidation of hexose-monophosphoric acid in presence of its dehydrogenase, the coenzyme II and a very small amount (0·02 mg.) of pyrocatechol acting as a carrier between polyphenol oxidase and the reduced coenzyme. The activity of the purest enzyme preparation obtained by Kubowitz was about 1000 times greater than that of the crude extract of potato peelings, and estimated by his method its Q_{O_2} is 30,000. The copper in more purified enzyme fractions was estimated by him manometrically from the rate of cysteine oxidation catalysed by ashed enzyme preparations. His purest enzyme fraction was thus found to contain 0·165 % Cu. Comparing this copper content with that of haemocyanine taken by Kubowitz to be about 0·34 %, he considers his purest preparation to be approximately 50 % pure.

(2) *Copper content of polyphenol oxidase preparations from mushrooms*

The amount of enzyme present even in our purest preparations was more than sufficient for direct estimations of copper by the diethyl-dithio-carbamate method leaving enough material for further work.

Copper estimation. The material is destroyed with sulphuric acid in a Kjeldahl flask heated over a small flame. When the combustion is complete, the fluid is diluted to 30 c.c., with glass distilled water, neutralized, mixed with 5 c.c. 40 % citric acid solution, and rendered alkaline with ammonia. The mixture is quantitatively transferred to a separating funnel to which is added 10 c.c. of 0·1 % aqueous solution of sodium diethyl-dithio-carbamate. The mixture, kept alkaline to litmus paper with ammonia, is immediately extracted with four lots (2 c.c. each) of carbon tetrachloride. The combined yellow extracts are transferred into the small cylinder of the Wolff colorimeter and compared with the colour of a carbon tetrachloride extract of a standard copper solution treated with sodium diethyl-dithio-carbamate in exactly the same way.

The weight of copper in the samples of material used for our estimations varied from 0·005 to 0·02 mg. Blank experiments carried out with all the reagents and all the manipulations involved in the estimation did not reveal any appreciable amount of copper in the reagents.

The results of copper estimations carried out on some enzyme fractions described in this paper and on some fractions of other preparations are given in Table I and figs. 1 and 2. These results clearly demonstrate that the concentration of Cu estimated as per cent. of dry weight increases with the rise in enzyme activity expressed in terms of purpurogalline number (P.N.) until it reaches a very high value of about 0·3 % for P.N. 940. This fact in itself strongly supports the general conclusion of Kubowitz that the active group of polyphenol oxidase contains copper. We could not, however, find a strict proportionality between the copper contents and activities in a series of preparations from P.N. – 0·65 to P.N. – 155. In this series we find only a general drift indicating an increase of copper content in more or less irregular steps, with the increased activities of the preparations. The amount of copper per unit of enzyme is at the beginning very high (137–350γ per E.U.); it then drops rapidly (to 6·8–25γ per E.U.) remaining still much too high in comparison with that of the pure sample.

The proportionality between the enzyme activity and copper content becomes apparent only in preparations above P.N. – 250 when the amount

TABLE I. RELATIONSHIP BETWEEN THE ACTIVITY (P.N.) AND COPPER CONTENT OF DIFFERENT ENZYME PREPARATIONS

P.N., purpurogalline number or mg. of purpurogalline formed in 5 min. from pyrogallol per mg. dry weight of preparation at 20° C. E.U., enzyme units or quantity of enzyme which forms 1000 mg. purpurogalline in 5 min. at 20° C.

Activity of the enzyme as P.N.	% Cu	γ Cu/E.U.
0·26	0·009	350·0
0·65	0·013	195·0
2·6	0·048	164·0
3·5	0·048	137·0
32	0·064	20·2
32	0·079	25·0
38	0·073	19·4
47	0·072	15·3
67	0·091	13·6
75	0·088	11·9
155	0·105	6·8
250	0·112	4·5
310	0·109	3·5
420	0·150	3·6
640	0·218	3·4
940	0·300	3·2

of copper per enzyme unit approaches a comparatively low and constant value of about 3·4γ.

It is somewhat difficult to determine the state of purity of our most active preparation (P.N. = 940). If Kubowitz's assumption that his purest preparation with copper content of 0·165 % is about 50 % pure enzyme, our preparation with copper content 0·30 % would be about 90 % pure. On the other hand, according to Hernler and Philippi (1930, 1933) the Cu content of various haemocyanines varies from 0·173 % (*Limulus*) to 0·26 % (*Loligo*).

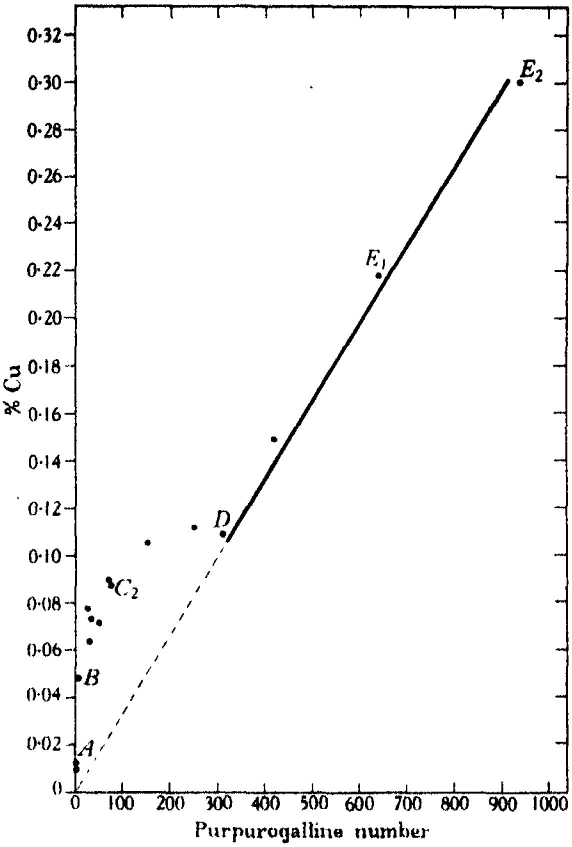

Fig. 1. Relationship between copper content and activity (P.N.) of different preparations of mushroom polyphenol oxidase. The letters (A–E_2) refer to the fractions described in this paper; remaining points refer to other enzyme preparations.

The copper content of our purest preparation is therefore higher than that of pure crystalline haemocyanine. It is conceivable that this preparation is already the pure enzyme.

In the first crude extract of mushrooms (P.N. = 0·65) the copper of polyphenol oxidase represents only about 1·6 % of the total copper present in this extract. The remaining copper does not seem to be in a dialysable state but bound to some of the proteins, and may in part belong to some other enzymes. It is also possible that polyphenol oxidase itself may exist in an inactive state which by some unknown process may be made active. This would explain an occasional increase of enzyme activity in a preparation kept for several days. The inactive enzyme (or proenzyme), if it does exist, cannot account, however, for more than a fraction of 1 % of the total copper of these crude preparations.

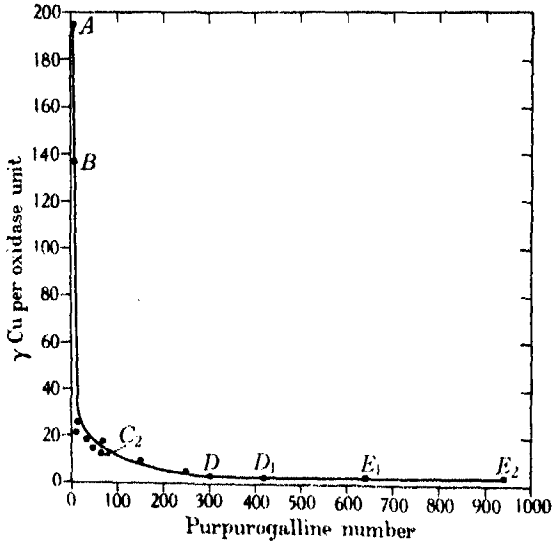

Fig. 2. The amount of copper (in γ) per enzyme unit in different preparations of mushroom oxidase. For letters see fig. 1.

It is only by successive steps of purification that the copper which does not belong to the polyphenol oxidase is gradually eliminated, together with other impurities.

One of the difficulties in comparing our results with those obtained by Kubowitz is due to the difference in methods of estimation of enzyme activity. While our method is based on the rate of oxidation of polyphenols, that of Kubowitz is based on the rate of oxidation of hexosemonophosphoric acid by its dehydrogenase system and catechol, the latter acting only as a carrier.

If the rate of reduction of catechol in this system is not the limiting factor of the reaction, the activity of potato oxidase would be at least twenty times lower than that of mushroom oxidase of the same copper content.

On the other hand, the activity of the purest preparation obtained by Kubowitz is about 1000 times higher than that of the crude extract of potato peelings. As the crude extract, estimated by our method, has P.N. of about 0·14–0·2, the activity of his pure preparation expressed in P.N. should be about 140–200. Estimated in this way the activity of potato oxidase would be still two or three times lower than that of mushrooms for the same amount of copper.

The difference in the activity of these two enzymes can only be explained by differences in their proteins. This is not surprising, however, considering that haemocyanines of different origin show very marked differences in the size of their molecules, their copper content and their oxygen binding properties.

It can be mentioned here that according to Yakushiji (1937), a haematin compound present in his "purified" preparation of polyphenol oxidase of *Lactarius piperatus* is probably the active grouping of this oxidase. This supposition was based, however, on an isolated observation, neither the amount of haematin was estimated nor the relationship between its concentration and the enzyme activity determined. Moreover, the purity of his "active" preparation ($Q_{O_2} = 11,280$) does not represent more than 1 % of our pure enzyme preparation. In such a crude preparation we have always found haematin which was, however, gradually eliminated during further purification. It is therefore highly probable that the active group of Lactarius polyphenol oxidase also contains copper.

5. PROPERTIES OF POLYPHENOL OXIDASE

The most remarkable property of this enzyme is its comparatively high specificity. Even the crude mushroom extract oxidizes, with great velocity, mainly the orthodihydroxyphenols, e.g. catechol, pyrogallol and dioxyphenylalanine but very few monophenols, e.g. *p*-cresol. The comparatively slow oxidation of tyrosine may be due mainly to its insolubility. On the other hand, the para compounds such as hydroquinone and *p*-phenylenediamine are scarcely oxidized (Table II and fig. 3). Moreover, the oxidations of catechol and *p*-cresol are strongly inhibited by CO (fig. 4) and this inhibition is not affected by light. In this respect the mushroom oxidase does not differ from the polyphenol oxidase of potatoes (Keilin 1929).

We may now ask whether the oxidation both of polyphenols and monophenols is catalysed directly by the same enzyme or whether the oxidation of monophenols and perhaps of some polyphenols requires the co-operation of an additional factor.

TABLE II. OXIDATION OF DIFFERENT SUBSTRATES BY CRUDE EXTRACT OF CULTIVATED MUSHROOMS; 0·2 C.C. EXTRACT (= 8 MG. DRY WEIGHT)

Substrates 10 mg.	*p*H	O_2 absorbed cu.mm./min.	$Q_{O_2} = \frac{\text{cu.mm. } O_2}{\text{1 hr. 1 mg.}}$
p-Cresol	7·3	47	320
Tyrosine	7·3	6	45
Hydroquinone	7·3	2	15
Hydroquinone	6	2	15
Hydroquinone	4·5	1	7
Catechol	7·3	78	585
"Dopa"	7·3	50	375
Pyrogallol	7	50	375

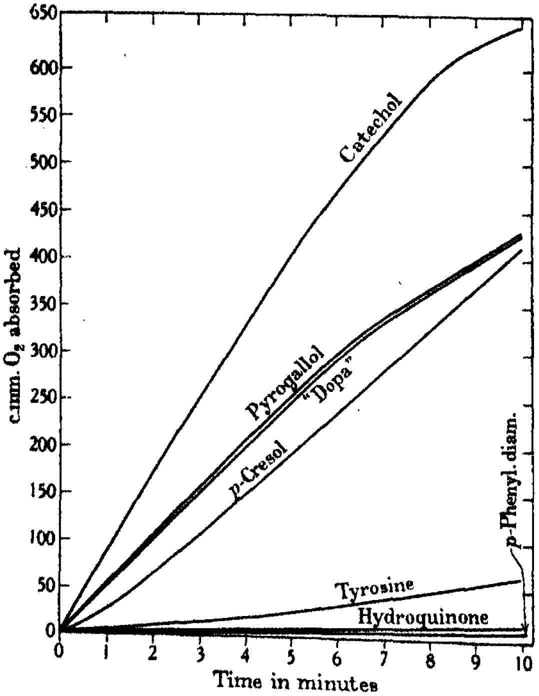

FIG. 3. Oxidation of various mono- and polyphenols by the crude extract of mushrooms; 0·2 c.c. of crude extract (= 8 mg. dry weight) 10 mg. substrate in 3·3 c.c. of M/30 phosphate buffer, *p*H 7·3; 20° C.

This question may partly be answered by comparing the rates of oxidation of catechol and *p*-cresol catalysed by enzyme preparations of different grades of purity. In doing so it is, however, important to bear in mind the fact that the oxidation of monophenols differs from that of polyphenols in having a more or less protracted lag period. The comparison is, therefore, made between the initial rate of oxygen uptake of the former and the somewhat delayed maximum rate of oxygen uptake of the latter.

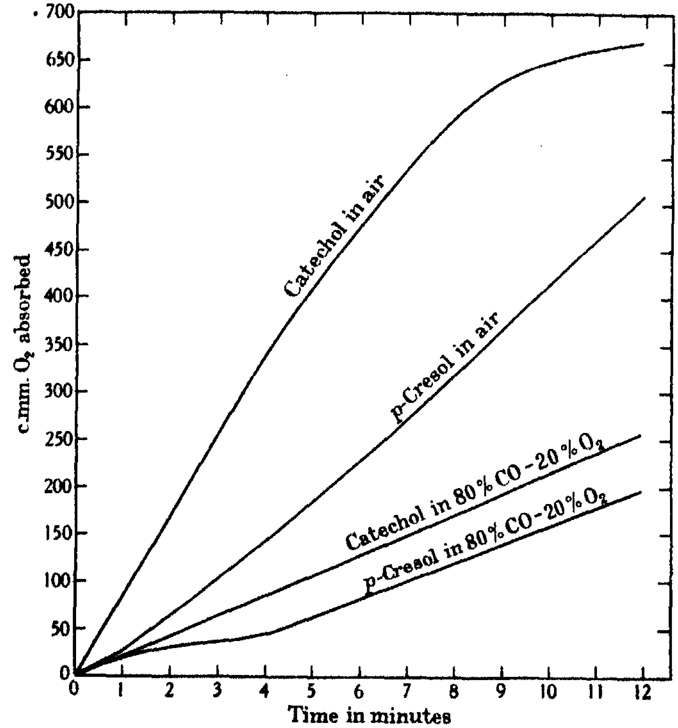

FIG. 4. Effect of CO on the oxidation of catechol and *p*-cresol by the crude extract of mushrooms; conditions as in fig. 3.

The experimental results show that the ratio between the rates of oxygen uptake by catechol and *p*-cresol is not constant. It increases already after the first lead acetate precipitation, the increase becomes even more apparent after the first adsorption on tricalcium phosphate and reaches a very high value with the purified enzyme (fig. 5).

These experiments reveal also two other peculiarities of the oxidation of *p*-cresol, namely: (1) on gradual purification of the enzyme the lag period becomes more protracted, and (2) pure enzyme catalyses the oxidation of *p*-cresol only when the enzyme is used in a very high concentration.

While catechol in presence of 0·6γ pure enzyme in 1 min. takes up 11·6 cu.mm. oxygen, *p*-cresol even in presence of 3·2γ enzyme does not take up any appreciable amount of oxygen. Only in presence of a much larger amount of pure enzyme, e.g. 32γ, does *p*-cresol undergo a slow oxidation taking up 13, 12 and 10 cu.mm. in three successive periods of 5 min.

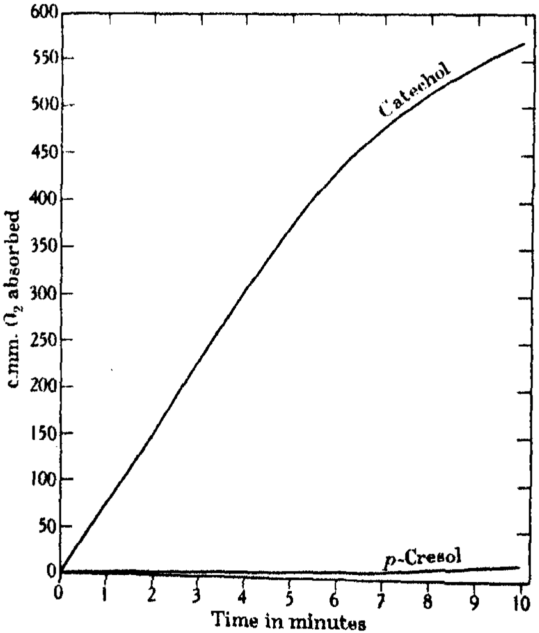

Fig. 5. Oxidation of catechol and *p*-cresol by the purified enzyme preparation. 5γ oxidase (containing $1{\cdot}5 \times 10^{-2}\gamma$ Cu); 5 mg. catechol or *p*-cresol in 3·3 c.c. M/30 phosphate buffer, *p*H 7·3; 20° C.

On purification the enzyme becomes gradually more specific for the oxidation of such compounds as catechol and pyrogallol, while its ability to oxidize other phenols gradually decreases (Table III).

Although we cannot yet offer a final explanation of these phenomena, our experiments suggest that polyphenol oxidase is a highly specific enzyme. Its catalytic activity seems to be restricted to a small group of polyphenols, while the oxidation of other substances especially the monophenols, probably requires the presence or the gradual formation of an

TABLE III. OXIDATION OF DIFFERENT SUBSTRATES BY PURE POLYPHENOL OXIDASE (P.N. = 940) FROM MUSHROOMS IN M/30 PHOSPHATE BUFFER *p*H 7·3; "DOPA" = DIOXYPHENYLALANINE

	Oxidase			
Substrates 10 mg.	Enzyme preparation	Copper content	O_2 uptake cu.mm./min.	$Q_{O_2} = \frac{\text{cu.mm. } O_2}{\text{mg. enzyme, 1 hr.}}$
Catechol	0·6γ	$1{\cdot}8 \times 10^{-3}\gamma$	11·6	1,160,000
	1·2γ	$3{\cdot}6 \times 10^{-3}\gamma$	20	1,000,000
	1·6γ	$4{\cdot}8 \times 10^{-3}\gamma$	27	1,012,000
	3·2γ	$9{\cdot}6 \times 10^{-3}\gamma$	54	1,012,000
"Dopa"	3·2γ	$9{\cdot}6 \times 10^{-3}\gamma$	6	120,000
	6·4γ	$1{\cdot}9 \times 10^{-2}\gamma$	9	85,000
Adrenaline	0·8γ	$2{\cdot}4 \times 10^{-3}\gamma$	3	225,000
	1·6γ	$4{\cdot}8 \times 10^{-3}\gamma$	6	225,000
	3·2γ	$9{\cdot}6 \times 10^{-3}\gamma$	9	170,000
	6·4γ	$1{\cdot}9 \times 10^{-2}\gamma$	12	110,000
Hydroquinone	12·8γ	$3{\cdot}8 \times 10^{-2}\gamma$	1	4,700
Pyrogallol	1·6γ	$4{\cdot}8 \times 10^{-3}\gamma$	19	710,000

additional factor, the nature of which remains still to be determined. That this additional factor may be a natural substance of the orthoquinone type has already been suggested by previous workers (Wheldale-Onslow 1931 and others). In fact, polyphenol oxidase, even when highly purified, can, in presence of a very small concentration of catechol, oxidize many substances, such as guaiacum, benzidine, reduced coenzyme, haemoglobin, etc. An interesting example of such an oxidation is that of ascorbic acid. This substance is not oxidised by catechol or by polyphenol oxidase alone, but when the three are brought together, ascorbic acid is rapidly oxidized (fig. 6). The oxygen uptake by this system is, moreover, strongly inhibited by carbon monoxide (fig. 7).

That ortho-quinones may play an important role in plant respiration acting as true respiratory carriers has already been suggested by Palladin (1910) and developed, among other workers, by Oparin (1921, 1927).

The recent work on the subject seems to give fresh support to this theory of plant respiration.

We wish to express our thanks to Dr E. F. Hartree for assistance in several experiments carried out in connexion with this study.

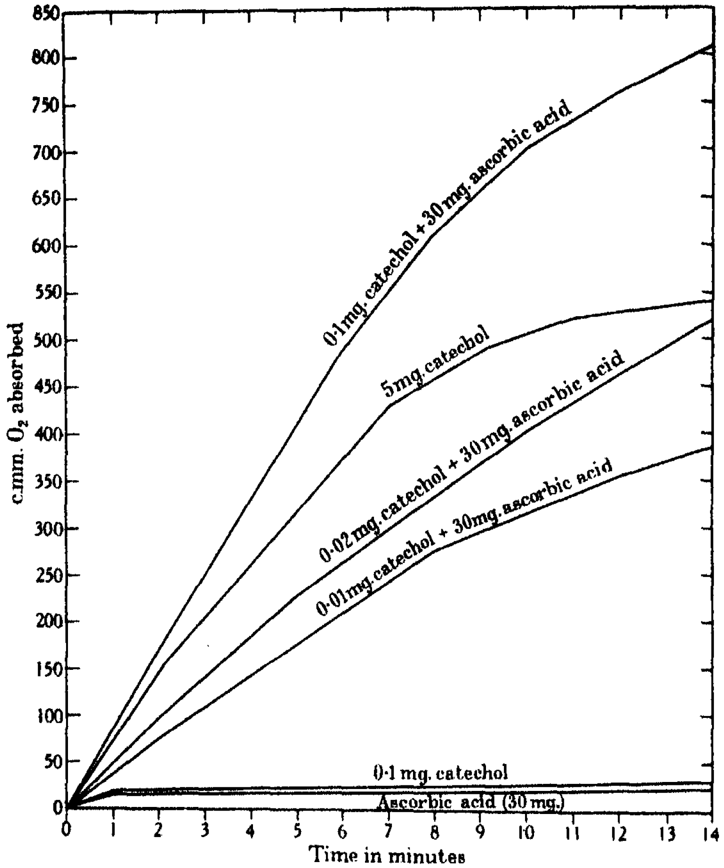

FIG. 6. Oxidation of ascorbic acid by purified mushroom oxidase in presence of a catalytic amount of catechol; 5γ enzyme containing $3 \times 10^{-3}\gamma$ Cu; catechol and ascorbic acid as shown in the figure; 3·3 c.c. M/30 phosphate buffer *p*H 7·3, 20° C.

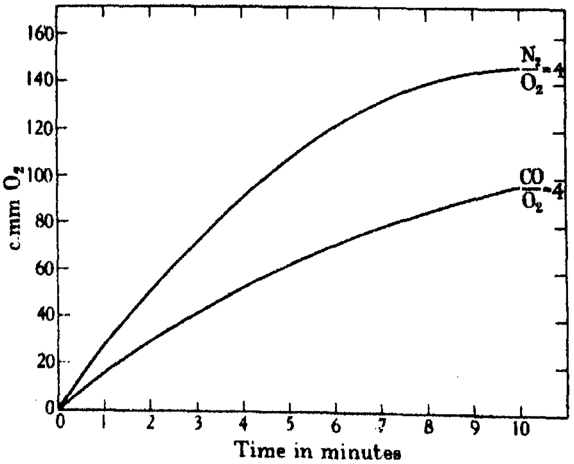

FIG. 7. Effect of CO on the oxidation of ascorbic acid by polyphenol oxidase and catechol; 1·6γ oxidase which contains $4{\cdot}8 \times 10^{-3}\gamma$ Cu; 30 mg. ascorbic acid; 0·02 mg. catechol; 3·3 c.c. M/30 phosphate, *p*H 7·3; 20° C.

6. SUMMARY AND CONCLUSIONS

1. A comparatively simple method of preparation of highly purified polyphenol oxidase from cultivated mushrooms (*Agaricus campestris*) is described.

2. The activity of the enzyme in different preparations was determined by two methods: (1) manometric, from the initial rate of oxygen uptake by catechol at 20° C., calculated per mg. dry weight per hour and expressed as Q_{O_2} and (2) colorimetric, from milligrams of purpurogalline formed from pyrogallol in 5 min. at 20° C./mg. dry weight and expressed as purpurogalline number (P.N.). The amount of enzyme which, under these conditions produces 1000 mg. purpurogalline is taken as one enzyme unit (E.U.).

3. The purest enzyme preparation obtained was completely free from haematin.

4. The polyphenol oxidase of mushrooms was found to be a copper-protein compound, the copper being a constituent of the active group of the enzyme. This extends the discovery of Kubowitz, who found that the corresponding oxidase in potatoes is a compound of copper with protein.

5. No proportionality between the copper content and the enzyme activity was found for preparations ranging from P.N. = 0·45 to P.N. = 250. These preparations contain large amounts of copper which does not belong to the enzyme. Within this range the amount of copper per enzyme unit (E.U.) gradually diminished from 196 to 7γ. Only when the copper content reaches a low and constant value of about 3·2–3·5γ per E.U. does the copper content become strictly proportional to the enzyme activity.

6. The purest enzyme preparation obtained has P.N. = 940, Q_{O_2} = 1,160,000 and copper content of 0·30 %. As this copper content is higher than that of pure crystalline haemocyanine, which may vary according to origin, from 0·173 to 0·26 % (Hernler and Philippi 1933), the enzyme may be considered as pure. 1γ Cu of this enzyme transfers to catechol, at 20° C. in 1 min., about 6000 cu.mm. O_2. 9·6 mg. of this enzyme (dry weight) was obtained from 15 kg. fresh mushrooms.

7. The crude extract of mushrooms readily oxidized only such compounds as catechol, pyrogallol and *p*-cresol. Other related compounds such as hydroquinone and *p*-phenylenediamine are hardly oxidized by this enzyme.

On purification, the enzyme gradually loses the property of catalysing the oxidation of monophenols.

8. All the oxidation reactions catalysed directly or indirectly by the polyphenol oxidase are inhibited by KCN, H_2S and CO.

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The coagulation of plasma by trypsin

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Introduction

During an investigation into the relation between the bactericidal power of serum and its antitrypsin content, Wright (1915) noted that the ability of blood to clot might be destroyed by the addition of a sufficient quantity of trypsin. Douglas and Colebrook (1916) confirmed this observation, further demonstrating that small concentrations of trypsin may have the reverse effect upon freshly drawn blood, namely, that of shortening the coagulation time. They did not analyse this phenomenon or propose an explanation for it. Both workers used a commercial pancreatic extract as the source of trypsin. Heard (1917) subsequently stated that trypsin clots oxalated plasma, but evidence has been produced (Mellanby 1935) to show that Heard's results are explicable as being due to the presence of small quantities of calcium in the commercial preparations which he also had used. Meanwhile, Waldschmidt-Leitz, Stadtler and Steigenwaldt (1928) had concluded that fibrinogen is acted upon by trypsin in the same manner as by thrombase and had suggested the identity of, or at least close similarity between, thrombase and trypsin.

Recently, Eagle and Harris (1937) have found that crystalline trypsin in suitable concentration coagulates the citrated plasma of the rabbit, guinea-pig, horse and man. According to them, trypsin does not coagulate fibrinogen, but activates prothrombase to thrombase in the absence of calcium.

Thus, although there is fairly general agreement that under certain circumstances preparations containing trypsin may accelerate the clotting of blood, it is still doubtful (*a*) whether the active agent is trypsin itself, (*b*) whether its action is directly upon fibrinogen or upon some part of the normal mechanism for coagulation, and (*c*) if the latter, whether it behaves as a thrombase, a thrombokinase, or in some other way.

The experiments presented here provide an analysis of the processes which take place when trypsin is added to plasma.

TECHNIQUE

The pancreatic juice used in the experiments was obtained from chloralosed cats, after cannulation of the pancreatic duct, by repeated injections of secretin, which had been prepared according to the method described elsewhere (Mellanby 1932). The later samples of pancreatic juice obtained by the use of secretin alone contain considerably less trypsinogen than the earlier samples, and if this form of stimulation be continued the trypsinogen content of the juice obtained falls almost to zero. Pancreatic juice rich in enzymes, including trypsinogen, may be secured by the subcutaneous injection of 0·66 mg. eserine salicylate 30 min. before the commencement of the experiment. This is followed 10 min. before the experiment by a second similar dose. Five minutes later the cat receives a subcutaneous injection of 2 mg. acetylcholine hydrochloride. This dose is repeated hourly for as long as the collection of pancreatic juice, stimulated by the injection of secretin, continues. Under these circumstances salivation is intense, and, in order to avoid the risk of asphyxia, it is necessary to insert a tracheal cannula. The enzyme content of the pancreatic juice so obtained varies somewhat in different cats, but 0·1 ml. of an average specimen, when activated by enterokinase, clots 2 ml. of calcified milk in less than 5 sec.

The source of enterokinase was pig's duodenal mucous membrane from which secretin had been prepared. It had been extracted with alcohol and dried after treatment with acetone. An aqueous extract of this material was capable of activating the trypsinogen in 100 times its own volume of pancreatic juice in 30 min.

For the experiments with fowl plasma, cockerels were bled, under urethane anaesthesia, into waxed centrifuge tubes, via a cannula tied into the sciatic artery. The blood was promptly centrifuged and the plasma so obtained kept at 0° C. in clean test-tubes until required for use. Such plasma is stable and shows no tendency to clot spontaneously.

The prothrombase and thrombase used were prepared according to methods already published (Mellanby 1930, 1933).

The *in vitro* experiments were carried out at 38° C.

EXPERIMENTAL

(1) *The action of trypsin on oxalated ox plasma: the digestion of fibrinogen*

We have obtained no evidence that activated pancreatic juice in any concentration clots oxalated ox plasma. Hence trypsin has no thrombase activity. Moreover, the addition of a sufficient quantity of activated pancreatic juice to oxalated ox plasma destroys the ability of the plasma to clot in the presence of added thrombase. This is seen in Table I, which shows the result of adding thrombase to samples of plasma which have been incubated with diluted activated pancreatic juice for varying intervals of time, and which showed no evidence of coagulation.

TABLE I

Oxalated ox plasma	H_2O	P.J. 1/10	Effect of 0·1 thrombase after				
			3 min.	5 min.	8 min.	10 min.	30 min.
0·1	0·4	0·5	N.C.	N.C.	N.C.	N.C.	N.C.
0·1	0·5	0·4	+	N.C.	N.C.	N.C.	N.C.
0·1	0·6	0·3	+	+	N.C.	N.C.	N.C.
0·1	0·7	0·2	+	+	+	+	+
0·1	0·8	0·1	+	+	+	+	+
0·1	0·9	0	+	+	+	+	+

N.C. = no coagulation. + = coagulation.
P.J. 1/10 = activated pancreatic juice, 1, + 0·85 % NaCl, 9.
All quantities in ml.

In this experiment, when the proportion of diluted activated pancreatic juice to plasma exceeds 2 : 1, incubation for a period of 8 min. suffices to abolish the coagulability of the sample; whereas, when that proportion is 2 : 1 or less, incubation for an indefinite period leaves intact at least sufficient fibrinogen to form a clot when thrombase is added. The loss of coagulability is due to the digestion of fibrinogen. That it is not due to the rapid digestion of the added thrombase is clear from the fact that when the proportion of diluted pancreatic juice to plasma is 4 : 1, addition of 0·1 ml. thrombase after 3 min. incubation produces a clot, whereas addition of thrombase to a second sample after 5 min. or longer period produces no clot (Table I). Moreover, the addition of excess thrombase to the latter sample produces no clot, but if fresh plasma be then added, a clot is rapidly formed, proving that free thrombase is present. The incoagulable sample therefore contained no fibrinogen.

The rate of digestion of fibrinogen, and therefore of loss of coagulability, depends upon the concentration of trypsin present and also upon the antitryptic activity of the plasma.

In the experiment quoted, the antitrypsin content of 0·1 ml. plasma was sufficient to neutralize the tryptic activity of 0·2 ml. diluted pancreatic juice. The addition of any greater quantity of pancreatic juice ultimately digested the fibrinogen.

(2) *The action of trypsin on (a) normal and (b) oxalated fowl plasma*

(*a*) Activated pancreatic juice coagulates fowl plasma, although it is unable to coagulate oxalated fowl plasma.

TABLE II

Fowl plasma	H_2O	P.J.	Coagulation time	Thrombase	Coagulation
0·5	0·5	0	N.C.	0·1	+
0·5	0·3	0·2	N.C.	0·1	N.C.
0·5	0·4	0·1	70 sec.		
0·5	0·4	0·1 P.J./2	50 sec.		
0·5	0·4	0·1 P.J./4	80 sec.		
0·5	0·4	0·1 P.J./8	130 sec.		
0·5	0·4	0·1 P.J./16	205 sec.		
0·5	0·4	0·1 P.J./32	340 sec.		
0·5	0·4	0·1 P.J./64	470 sec.		

As Table II shows, there is an optimal concentration of pancreatic juice at which coagulation occurs most rapidly, whilst concentrations above and below this are less effective. When sufficient pancreatic juice is added, the plasma is rendered incoagulable even on the addition of excess thrombase. At least two, and probably three, processes are involved:

(i) The coagulating effect varies with the concentration of pancreatic juice.

(ii) Digestion of fibrinogen proceeds at the same time. When enough trypsin is added, it is sufficiently rapid to prevent coagulation entirely.

(iii) Thrombase is probably destroyed also, although there is no direct evidence of such in this experiment.

(*b*) In view of the contrast between the effect of activated pancreatic juice upon oxalated ox plasma and normal fowl plasma, it is necessary to observe its action upon oxalated fowl plasma. For this purpose, 0·1 % potassium oxalate was added to fowl plasma and the above experiment repeated. The relevant part of the result is recorded in Table III.

Table III

Oxalated fowl plasma	H_2O	P.J.	Coagulation time	Thrombase	Coagulation
0·5	0·5	0	N.C.	0·1	+
0·5	0·4	0·1 P.J./2	N.C.	0·1	N.C.
0·5	0·4	0·1 P.J./4	N.C.	0·1	N.C.
0·5	0·4	0·1 P.J./8	N.C.	0·1	+
0·5	0·4	0·1 P.J./16	N.C.	0·1	+

Activated pancreatic juice does not clot oxalated fowl plasma. It will be observed that as little as 0·1 ml. of 25 % pancreatic juice is now able to destroy the fibrinogen of 0·5 ml. oxalated plasma, whereas in the presence of calcium ions this quantity of juice brought about coagulation in 80 sec. (Table II). It is evident that the formation of thrombase by trypsin, whatever the process involved, is much more rapid than the digestion by trypsin of fibrinogen. The addition of 0·1 % sodium citrate to fowl plasma similarly prevents coagulation by trypsin. Fibrinogen digestion takes place to the same extent as in oxalated fowl plasma. This is in marked contrast with the results obtained by Eagle and Harris (1937).

The effect of activated pancreatic juice upon fowl plasma is due to the direct or indirect action of trypsin itself. This is shown by adding unactivated pancreatic juice and the enterokinase solution separately to the plasma. Neither alone produces coagulation, but when enterokinase is added to plasma containing unactivated pancreatic juice, either coagulation or digestion of fibrinogen occurs. Such an experiment is illustrated in Table IV.

The pancreatic juice in this case was from the same sample as in the two foregoing experiments (Tables II and III). The coagulation times here are much greater than in Table II, as would be expected, on account of the additional time required for the preliminary activation of trypsinogen.

Table IV

Plasma	H_2O	U	Coagulation time	E	Coagulation time	Thrombase	Coagulation
0·5	0·5	0	N.C.	0·1	N.C.	0·1	+
0·5	0·2	0·3	N.C.	0·1	N.C.	0·1	N.C.
0·5	0·3	0·2	N.C.	0·1	N.C.	0·1	N.C.
0·5	0·4	0·1	N.C.	0·1	320 sec.		
0·5	0·4	0·1 U/2	N.C.	0·1	233 sec.		
0·5	0·4	0·1 U/4	N.C.	0·1	245 sec.		
0·5	0·4	0·1 U/8	N.C.	0·1	285 sec.		
0·5	0·4	0·1 U/16	N.C.	0·1	385 sec.		
0·5	0·4	0·1 U/32	N.C.	0·1	525 sec.		
0·5	0·4	0·1 U/64	N.C.	0·1	1170 sec.		

U = unactivated pancreatic juice. E = solution of enterokinase.
E was added 20 min. after U.

Boiled pancreatic juice, before or after the addition of enterokinase, does not bring about the coagulation of fowl plasma, nor does it digest fibrinogen. Unactivated pancreatic juice does not digest fibrinogen.

The coagulation of normal, but not of oxalated, plasma by trypsin suggests either that trypsin has the same function with respect to the process of coagulation as thrombokinase, or that thrombokinase is formed by the action of trypsin upon some constituent of the plasma. This question is dealt with below (§§ 3 and 4).

(3) *Activation of prothrombase by trypsin*

If trypsin is equivalent to a mixture of thrombokinase and calcium, activated pancreatic juice ought to activate prothrombase. To test this the following solutions were made up:

2 ml. prothrombase solution (1 mg. per ml.) + 0·2 ml. P.J. = A
2 ml. ,, ,, ,, + 0·2 ml. P.J./2 = B
2 ml. ,, ,, ,, + 0·2 ml. P.J./4 = C
2 ml. ,, ,, ,, + 0·2 ml. P.J./8 = D
2 ml. ,, ,, ,, + 0·2 ml. P.J./16 = E

These solutions were then incubated and tested for thrombase activity at 5 min. intervals. For this purpose 0·1 ml. of the solution was added to 0·4 ml. H_2O + 0·5 ml. oxalated ox plasma. At the end of 1 hr. no activation had taken place. The addition of a suitable quantity of $CaCl_2$ and thrombokinase to each tube then produced prompt activation in D and E, indicating that the prothrombase was intact, but no activation in A, B, and C, showing that in these solutions the concentration of trypsin had been high enough to digest the prothrombase. Trypsin does not, therefore, activate prothrombase in the absence of calcium. On repeating the experiment with the addition of 0·02 ml. $N/2$ $CaCl_2$ to each solution, the result was unchanged; that is, trypsin cannot activate prothrombase even in the presence of ionized calcium.

(4) *The action of trypsin on thrombokinase-free plasma*

If trypsin is not itself thrombokinase, but merely renders active thrombokinase which was already present, it should be possible, by removing from fowl plasma its hidden kinase, to prepare a plasma in which coagulation should ensue upon adding a thrombokinase extract, but not on the addition of activated pancreatic juice. This condition has been produced by keeping fowl plasma for several weeks at 0° C. Such plasma slowly deposits a scanty white precipitate which ultimately contains all the hidden thrombokinase.

Six ml. fowl plasma which had been kept for 7 weeks at 0° C. was centrifuged and the precipitate digested with 50% activated pancreatic juice. The resulting solution = D in the table below. The effects upon the supernatant plasma of a suspension of thrombokinase (testis extract) (= K), of the digest D, and of varying quantities of pancreatic juice are compared in Table V.

TABLE V

Plasma	H_2O	Coagulating agent	Coagulation time	Thrombase	Coagulation
0·5	0·4	0·1 P.J./2	N.C.	0·1	N.C.
0·5	0·4	0·1 P.J./4	N.C.	0·1	N.C.
0·5	0·4	0·1 P.J./8	N.C.	0·1	N.C.
0·5	0·4	0·1 P.J./16	N.C.	0·1	+
0·5	0·4	0·1 P.J./32	N.C.	0·1	+
0·5	0·4	0·1 K	65 sec.		
0·5	0·4	0·1 D	50 sec.		

Thrombose added 3 hr. after P.J. etc.

The fact that thrombokinase coagulates this plasma whereas trypsin does not, is further evidence that the coagulating action of trypsin depends upon the liberation of thrombokinase. It also strongly indicates that the thrombokinase responsible for the coagulation of fresh fowl plasma when activated pancreatic juice is added to it is derived from the plasma itself, and not from the pancreatic juice. In addition, it may also throw light on the relation between prothrombase and thrombokinase in plasma. From the fact that prothrombase precipitated in the usual way has associated with it some thrombokinase, possibly in a relatively inactive condition, it might be inferred that thrombokinase and prothrombase are normally attached to one another in plasma. If that is so, the bond between them is easily broken, for in the present experiment all the kinase has been precipitated, leaving in solution at least sufficient prothrombase to furnish enough thrombase to clot the plasma when active thrombokinase is added. It seems more likely that they are not attached to one another in any way, but that the usual conditions for the precipitation of prothrombase happen to be those for the precipitation of some thrombokinase.

(5) *The liberation of thrombokinase from fowl serum by trypsin*

Activated pancreatic juice acquires an enhanced coagulating action upon fowl plasma when treated with fowl serum, although the serum itself has no apparent coagulating effect. This phenomenon becomes significant in view of the thrombokinase-liberating action of trypsin on fowl plasma. In Table VI the coagulation times of fowl plasma with trypsin and a mixture of trypsin and serum are compared. Equal quantities of serum (S) and diluted activated pancreatic juice were incubated together for 5 min. before testing the thrombokinase activity of the mixtures.

TABLE VI

Plasma	H_2O	Coagulating agent	Coagulation *time* sec.
0·5	0·45	0·05 S	N.C.
0·5	0·45	0·05 P.J./2	295
0·5	0·4	0·1 (P.J.+S)	85
0·5	0·45	0·05 P.J./4	235
0·5	0·4	0·1 (P.J./2+S)	120
0·5	0·45	0·05 P.J./8	310
0·5	0·4	0·1 (P.J./4+S)	230
0·5	0·45	0·05 P.J./16	345
0·5	0·4	0·1 (P.J./8+S)	285
0·5	0·45	0·05 P.J./32	435
0·5	0·4	0·1 (P.J./16+S)	370

It is evident that, particularly with the larger concentrations of activated pancreatic juice, thrombokinase has been liberated in the serum. No such effect could be demonstrated with ox serum obtained from defibrinated blood. The plasma from which the fowl serum was obtained had clotted spontaneously, and very slowly, at 38° C. Under these conditions, the processes concerned with the activation of some prothrombase and the removal of fibrinogen from the fowl plasma do not lead to the removal of all the inactive thrombokinase which can be released by trypsin.

Discussion

The production of a tryptic ferment in serum shaken with chloroform was described by Jobling and Petersen (1914). Dale and Walpole (1916), investigating another observation of Jobling's, found that similar treatment of fowl plasma causes the liberation therein of thrombokinase. The latter workers were led to expect that trypsin would release thrombokinase in fowl plasma. Although they found difficulty in demonstrating the coagulant action of trypsin upon fowl plasma itself, they were able to produce some evidence that trypsin does in fact liberate thrombokinase in fowl plasma. Their finding in this respect is corroborated by the present experiments. They noted that chloroform may not release thrombokinase in plasma which had been kept some time, although it had produced the expected effect on fresh plasma. This, too, agrees with the results here presented, though the explanation for the phenomenon offered by Dale and Walpole is probably in error. They assumed that, on standing, the masked trypsin originally present in the plasma underwent decay, and could not, therefore, be revealed by the destruction, by chloroform, of antitrypsin. It has been shown, however, that old fowl plasma contains no hidden thrombokinase, so that even if trypsin were present, no activation of prothrombase could take place. Dale and Walpole do not describe any observations on the effect of adding trypsin to a sample of fowl plasma which has aged sufficiently to be incoagulable with chloroform.

It is probable that the action of trypsin in liberating thrombokinase is similar in nature to the generation of "metathrombin" in fowl serum by the addition of acid or alkali, with subsequent neutralization. It has previously been shown (Mellanby 1908) that "metathrombin" is thrombokinase.

In view of the hypothesis recently put forward by Eagle (1937), that trypsin and other proteolytic enzymes may replace calcium and platelets in the activation of prothrombase, it is necessary to discuss in some detail the relation of trypsin to the activation of prothrombase. The inference from Eagle's hypothesis is that thrombokinase is a proteolytic enzyme, active in the presence of calcium, whilst trypsin activates prothrombase in the absence of calcium. As has been demonstrated, such activation only occurs in the presence of calcium and when thrombokinase is available. Not only is there no direct evidence that thrombokinase is a proteolytic enzyme, but the proposal requires that antitrypsin should also neutralize thrombokinase, which it does not do. Were thrombokinase (with calcium) interchangeable with trypsin so far as the activation of prothrombase is concerned, it would be difficult to explain the coagulant action upon fowl plasma of amounts of trypsin far smaller than can be neutralized by the quantity of antitrypsin present in the plasma. It has been shown that the liberation by trypsin of thrombokinase is extremely rapid and the explanation offered for the coagulation of fowl plasma under the circumstances indicated is, that antitrypsin and inactive thrombokinase compete for the trypsin, which is able to liberate a small amount of thrombokinase before being completely inactivated by the antitrypsin. The plasma then slowly clots, although there is no free trypsin present in the solution.

CONCLUSIONS

1. Fowl plasma and serum contain an inactive form of thrombokinase.
2. Trypsin liberates active thrombokinase in fowl plasma and serum.
3. Trypsin therefore brings about the coagulation of fowl plasma.
4. Trypsin digests fibrinogen, thrombase, and prothrombase.
5. Trypsin activates prothrombase only in the presence of thrombokinase, which it liberates, and calcium.

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Studies on the hypophysectomized ferret
X. Growth and skeletal development

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[Plates 6–8]

The relation between the pituitary gland and body growth has been studied by means of hypophysectomy in a number of species of animals. Cushing (1911), from his results with hypophysectomy of the dog, concluded that the gland secreted a substance that controlled and maintained proportionate growth. The results of Smith (1927) and Collip (1934) and his co-workers on the rat show that there is little growth after removal of the pituitary, although the latter authors find that if the rat is hypophysectomized at an early age (body weight 25–35 g.) it continues to grow until a body weight of 60 g. is reached. The growth stasis following hypophysectomy seems to be characteristic of all mammals.

A detailed radiographical study of the effect of hypophysectomy on the development of bone in the rat has recently been made by Mortimer (1937). He finds that the degree of vascularity of the bone is greatly reduced, so that the resorption and deposition of calcium is lessened. Changes in the skeletal system are described as "an osteoporosis simplex with disorganization of the trabecular structure of bone and marrow aplasia". Special reference is made to the skull, since cranial growth, particularly antero-posterior growth, is greatly retarded by hypophysectomy.

Attempts have been made to ascertain which type of cell in the anterior lobe of the pituitary secretes the substance controlling growth. Evidence from clinical cases of pituitary tumours suggests that the eosinophil cells are concerned with this function of the pituitary. Further light has been thrown on this subject by Kemp's (1934) work on the hereditary dwarf mouse, the condition of which seems to be due to pituitary deficiency. Histological study of the pituitary of this dwarf shows a complete absence of eosinophil cells.

Further evidence of the role of the pituitary in normal body growth is shown by the increased skeletal effects that can be obtained by the administration of anterior pituitary extracts (Evans and Long 1921; Collip 1934; and Silberberg 1936). Evans and his co-workers (1933), however, were unable to obtain marked skeletal abnormalities by this means in the normal dog, although in the achondroplastic breeds (*Dachshunde*) they were able to produce symmetrical giantism. In the hypophysectomized animal, growth and skeletal development can be maintained or restored by the use of extracts, as shown by Smith (1927), Collip (1934) and by Evans and his co-workers (1933, 1935). The degree of purity of the extracts seems to be of importance since both Evans and Collip have shown that with purified extracts continuous growth is not maintained. The former author was able, on the other hand, to produce indefinite growth by the use of crude pituitary extracts and to show that the rats resistant to the purified extracts responded to the crude preparations. The results of Mortimer (1937) suggest that although crude extracts produce massive growth of a poorly differentiated type, concentrated extracts produce growth which resembles more closely the normal structure of bone.

MATERIAL

Six young male and two young female ferrets of 500–600 g. were hypophysectomized by the parapharyngeal route described by Hill and Parkes (1932). Three of the males and the two females were born in the laboratory, the dates of birth being recorded. The remaining three males were purchased from dealers so that the exact age was not known. Control material consisted of five males and four females, the exact age being known in all except one male and one female.

The animals were weighed twice weekly while growing rapidly; subsequently weighings were made once weekly. The weights were recorded at a constant time relative to feeding. The sella turcica was examined histologically for pituitary fragments if there was any doubt as to the completeness of the operation. During the breeding season the condition of the external reproductive organs is an indication of the completeness of the operation.

In assembling the data on growth in the ferret, males and females have been grouped separately on account of the difference between the two sexes. Figs. 1 and 3 show the increase in weight with increasing age over intervals of 10 days. The arrow in each figure indicates the time at which the animals in each group were hypophysectomized. In these figures only animals that were born in the laboratory are included.

GROWTH

Growth of the normal ferret. The ferrets were first weighed at 1 month old, when they all weighed 170–190 g., there being little sex difference. It will be seen from figs. 1 and 3 that the growth rate in the two sexes is the same until the end of the second month, when the males begin to grow more rapidly than the females. In the females the adult body weight is reached in about 4 months' time, while the males continue to increase in weight for another month. The adult weight of the female ferret is about 1 kg., while the male is nearly double this weight. The largest male handled in this work attained a maximum weight of 2·6 kg. in the space of 6 months (fig. 2).

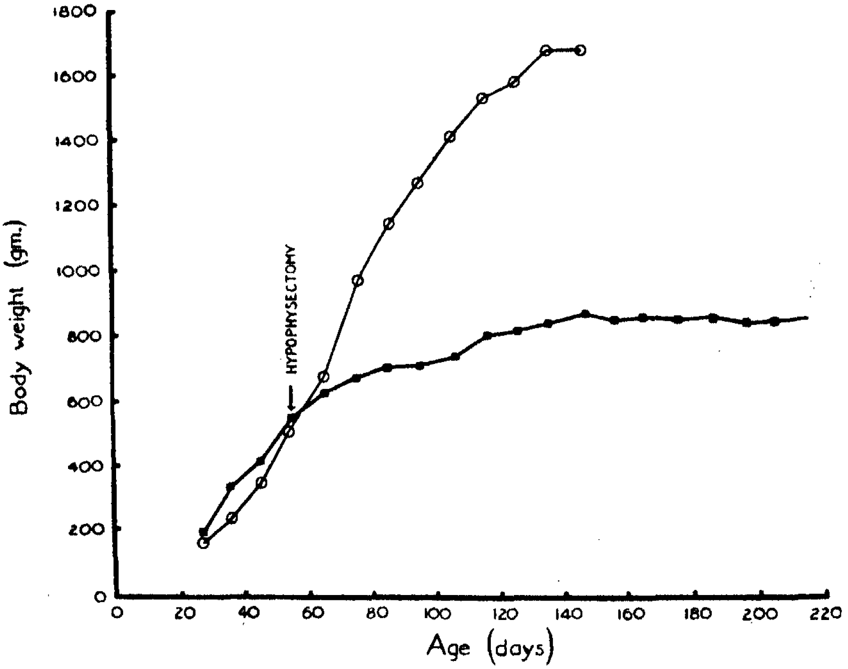

FIG. 1. The effect of hypophysectomy on the growth rate of the male ferret. The mean body weight of three hypophysectomized ferrets of known age (■) is compared with that of three normal male ferrets (o).

Growth of the ferret under laboratory conditions is not influenced by the date of birth; those born in August and September, when non-pregnant adults are entering anoestrus, grow throughout the autumn and winter at the same rate as ferrets born in June. It would appear, therefore, that the pituitary secretion responsible for the maintenance of growth is not subjected to seasonal variation such as is found in the case of the secretions regulating the gonads and the thyroid gland. In this respect the ferret under laboratory conditions is different from British mammals in the wild state.

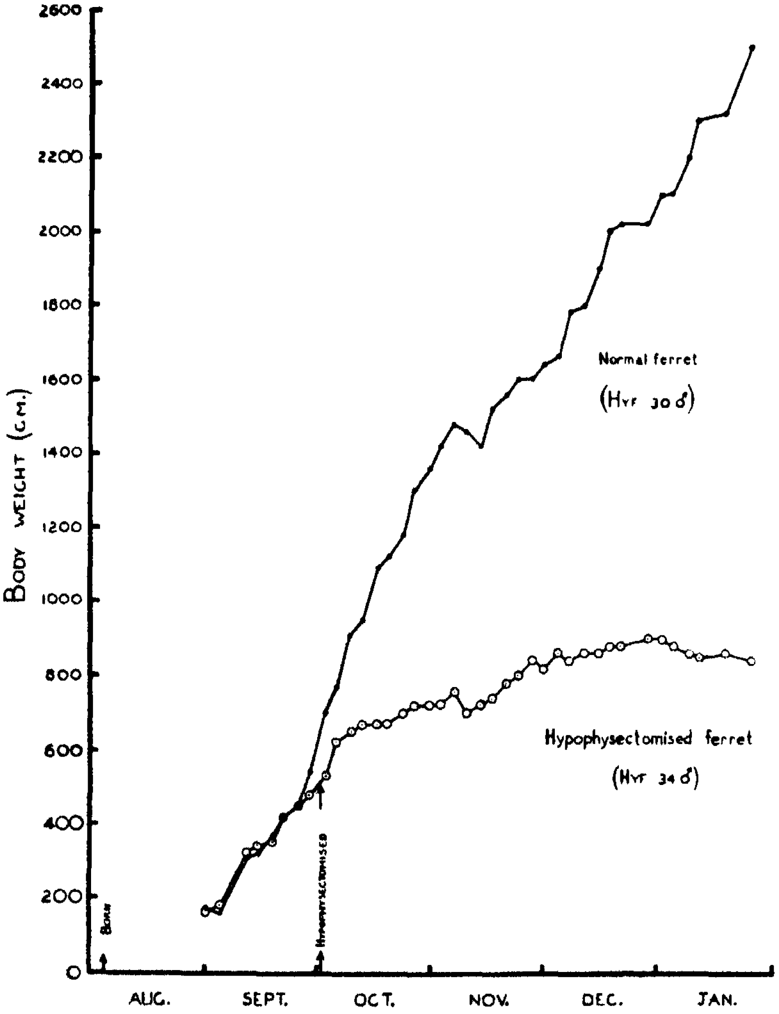

Fig. 2. The body weight of a hypophysectomized male ferret, HYF 34 (o), compared with that of normal litter-mate control, HYF 30 (●).

The growth of the hypophysectomized ferret. In the male ferrets of known age, hypophysectomy was performed at 51, 53 and 58 days respectively, and in the females at 67 and 72 days. Following the operation, the growth rate in the two sexes was greatly retarded. In the male, growth continued slowly until the age when growth in the normal ferret ceases. During this time, about 3 months, the maximum increment was approximately 300 g., as compared with 1200 g. for the normal animal. The male ferret, therefore, hypophysectomized at about 2 months old, is only half the normal weight at 4 months old (fig. 1).

Fig. 2 shows the growth rate of a male ferret hypophysectomized when 500 g., in comparison with that of a litter mate control. Both of these animals were fed on a similar diet and housed under identical conditions. They both lived for about 2½ years. The hypophysectomized one thus survived operation for over 2 years. He remained in good health and appeared normal except for his small size and failure to show enlargement of the testes during either of the two breeding seasons through which he lived. At autopsy, 16 November 1936, the reproductive organs were infantile, the weight of the os penis being only 70 mg. as compared with 420 mg. in the control litter mate. The thyroids and adrenals were very atrophic. No other pathological symptoms were noticed.

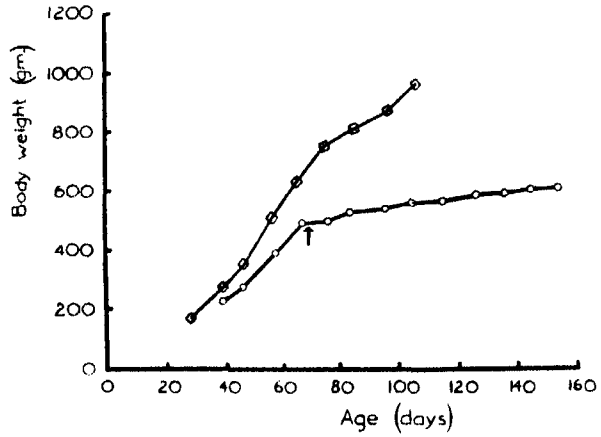

FIG. 3. The effect of hypophysectomy on the growth rate of the female ferret. The mean body weight of two hypophysectomized ferrets (o) is compared with that of three normal female ferrets (⊡).

The rate of growth in the females following hypophysectomy was slower than in the males, but it is proportional in comparison with the normal growth rate. The maximum increase in weight following the operation was about 100 g., which is attained in three months (fig. 3).

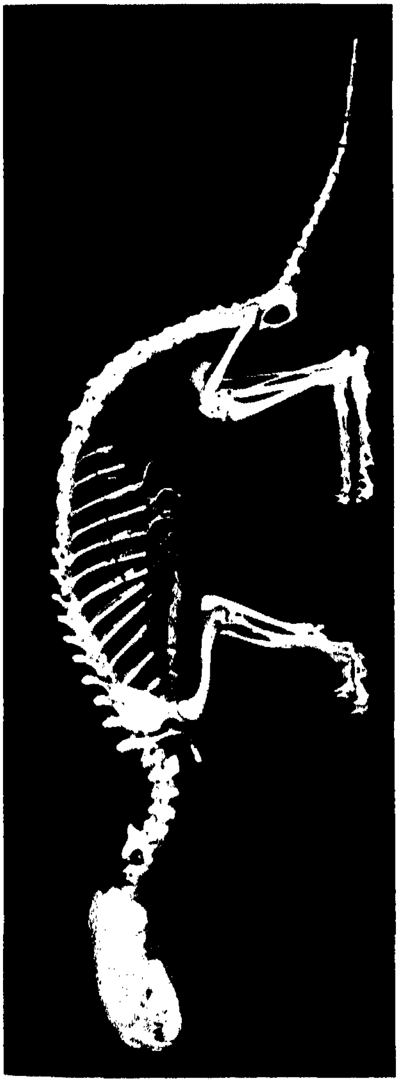

Fig. 5

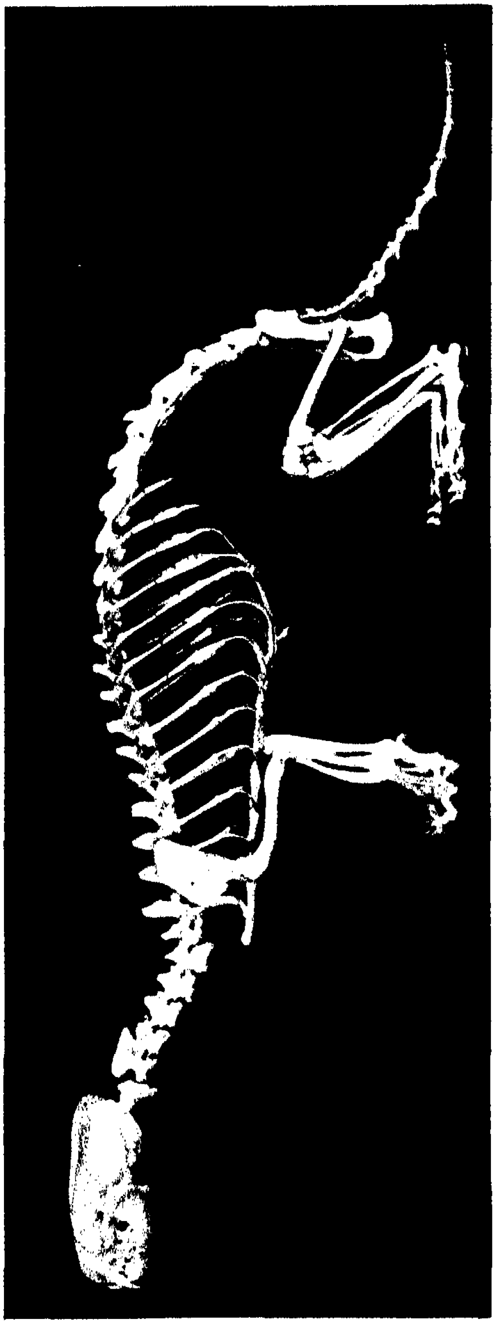

Fig. 4

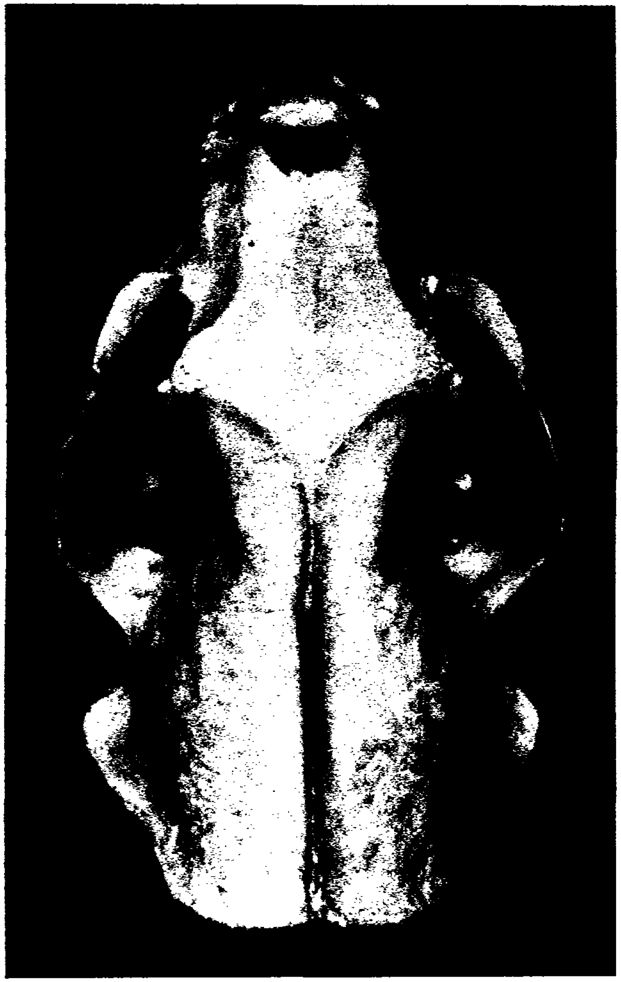

Fig. 6

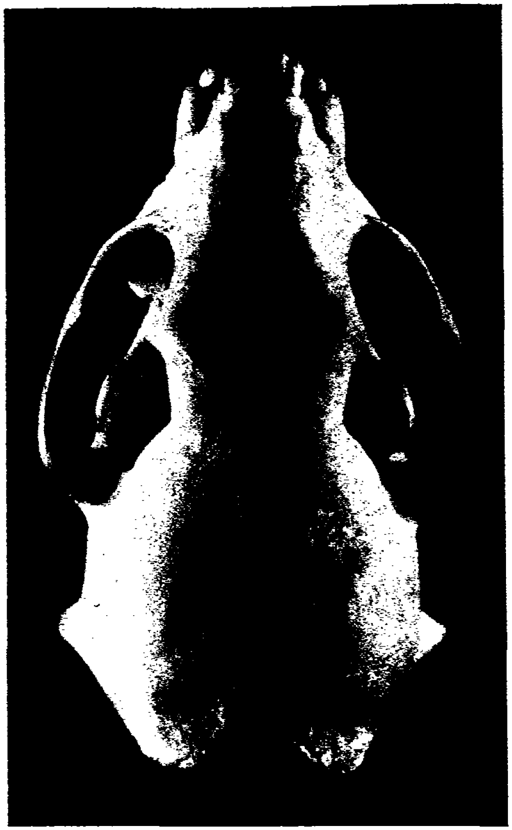

Fig. 7

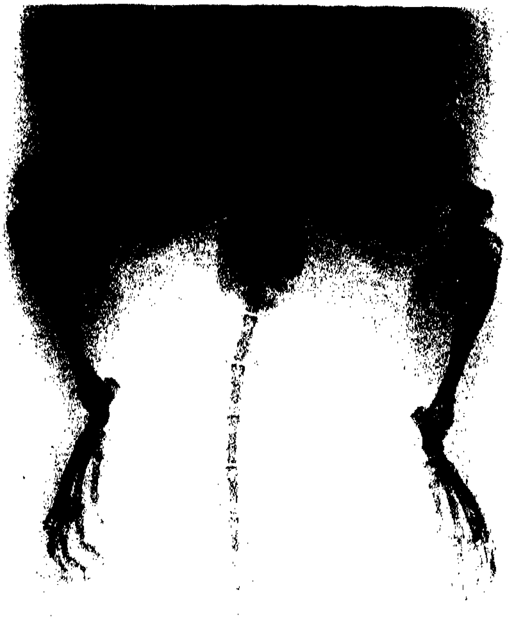

Fig. 8

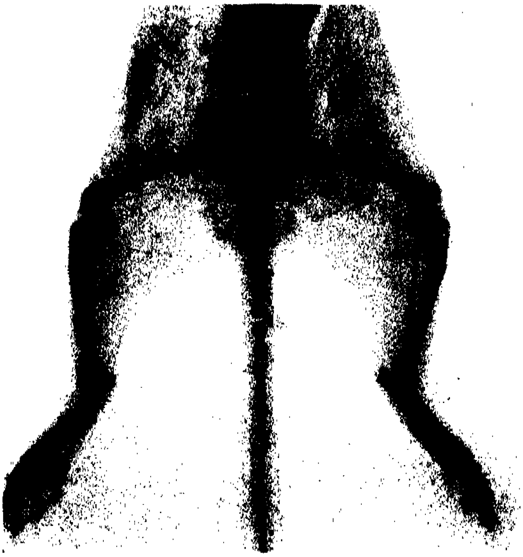

Fig. 9

These results, on the whole, are similar to those described for other species. In the males there is a great difference in weight between the normal and the hypophysectomized animal, while in the female, although the difference is not so pronounced, the curve obtained in the early stage following hypophysectomy is much flatter. The hypophysectomized ferret would thus be a good test animal for growth hormone.

SKELETAL DEVELOPMENT

Study of the general skeleton, chiefly by X-ray photographs and prepared skeletons of the two ferrets whose body weights are plotted in fig. 2, shows that in the hypophysectomized ferret (HYF 34) there is a general stasis in the growth of bone so that the skeleton (fig. 5, Plate 6), although the animal is adult and 2½ years old, resembles that of an immature ferret 4–5 months old. The various processes and crests of the bones which develop with age are only very slightly developed. These are particularly well shown in the skull, in the spinous processes throughout the vertebral column, and in the scapulae. None of the smaller prominences and depressions characteristic of the surfaces of adult bones, associated with the development of muscles, appear; they retain the smooth rounded appearance of the bones of an immature skeleton.

Ossification is arrested in the hypophysectomized ferrets as shown by the open epiphyses of the long bones and the vertebrae (fig. 9, Plate 8), and in the degree of ossification of the pubic symphysis. This, however, has not led to any undue lengthening of the bones; it is seen from Table I that growth in length of bone is retarded, the length of skeleton of the hypophysectomized ferret being about 20% shorter than that of the litter-mate control. The ratio of the length of humerus to radius in these two ferrets seems to be disturbed by retardation of the growth of humerus in the hypophysectomized ferret and by the continued growth of the radius. This, however, is of doubtful significance. There is a marked decrease in the calcium content, which is particularly well seen at the ends of the long bones and in the cartilaginous parts of the ribs. There is some softening of the bones due to the low calcium content, which in turn has given rise to slight bending in some.

Skeletal differences between the skulls of normal and hypophysectomized ferrets are well marked (figs. 6, 7, Plate 7). In the young ferret of about 2 months of age all the cranial sutures are open and there is no development of the fronto-parietal and nuchal crests. At about 6 months of age, shortly after the animal has reached its maximum body weight, rapid

TABLE I. COMPARISON OF SKELETAL DIMENSIONS OF NORMAL AND HYPOPHYSECTOMIZED FERRET

Dimension	Ferret Normal HYF 30 cm.	 Hypophysectomized HYF 34 cm.
Total length of skeleton	57·4	46·2
Length of tail	16·2	13·0
Ratio humerus to radius	4·6 : 2·5	3·8 : 2·75
Skull:		
Overall length	6·8	5·8
Width across zygomatic arches	4·5	3·7
Width between mastoid processes	3·9	3·4
Width between supra-orbital processes	2·5	1·9
Weight of skull	16·35 g.	10·08 g.

calcification of bone occurs, as shown by the process of fusion which is taking place in the skull, and by the simultaneous development of the sagittal and nuchal crests. In the adult ferret, 1 year or more, the fusion of the sutures is complete. In ferret HYF 34, 2 years after hypophysectomy, most of the bones of the skull were distinct and separate. The parietals and the nasals have open sutures and the basilar-sphenoid junction remained open. Partial fusion, beginning posteriorly, of the frontal bones, has occurred. The fronto-parietal suture was open, and there was no formation of a sagittal ridge. The appearance of this and other skulls from ferrets hypophysectomized at about 2 months of age was similar to those of ferrets of approximately 4–5 months old. Complete fusion of the sutures and the full development of the sagittal and nuchal crests had been permanently inhibited.

The difference in size of the skulls of these two ferrets can be seen by the measurements given in Table I. The retardation in width of skull is somewhat greater than that of length. The difference in the extent of ossification of the skulls can be judged by their difference in weight, which amounts to over 30%.

We wish to thank Mr T. A. Webster for taking X-ray photographs of some of these ferrets during the course of this work, and also Dr Meyer, St Bartholomew's Hospital, for taking the X-ray photographs shown on Plate 8.

Summary

Six immature male, and two immature female ferrets were hypophysectomized at a body weight of 500–600 g. (7–10 weeks of age). Subsequent body growth in ferrets of both sexes was retarded; in the female the stasis was almost complete, but the males continued to grow slowly until the age when the growth in the normal ferret is complete.

Skeletal development was studied by means of X-ray photographs and prepared skeletons. It was found that skeletal growth had been arrested; the bones had a low calcium content and open epiphyses. Stasis in the development of the skull was most marked, this being shown by the absence of sagittal and nuchal crests, open sutures and the retention of all the characteristics of the skull of an immature animal.

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Description of Plates 6–8

Plate 6

Fig. 4. Photograph of skeleton of normal adult male ferret (HYF 30), 2½ years old. × $\frac{2}{5}$.

Fig. 5. Photograph of skeleton of hypophysectomized male litter-mate ferret (HYF 34), 2½ years old. Hypophysectomy was performed at the age of 2 months. × $\frac{2}{5}$.

Plate 7

Fig. 6. Photograph of skull of normal ferret (HYF 30), showing the development of the sagittal and nuchal crests and the complete fusion of all sutures. × $\frac{5}{3}$.

Fig. 7. Photograph of skull of hypophysectomized ferret (HYF 34), showing the preservation of characters of the skull of an immature ferret. × $\frac{5}{3}$.

Plate 8

Fig. 8. X-ray photograph of posterior region of normal ferret (HYF 30). × ½.

Fig. 9. X-ray photograph of posterior region of hypophysectomized ferret showing the open epiphyses of the caudal vertebrae. × ½.

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Molecular structure in relation to oestrogenic activity. Compounds without a phenanthrene nucleus

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From the Courtauld Institute of Biochemistry

(*Communicated by Sir Henry Dale, F.R.S.—Received* 11 *December* 1937)

In earlier publications the oestrogenic activity of a number of synthetic substances has been discussed. The first compounds to be tested contained the phenanthrene ring system, and it was shown (Cook, Dodds and Hewett 1933; Cook and Dodds 1933; Cook, Dodds, Hewett and Lawson 1934; Cook, Dodds and Greenwood 1934) that a high degree of activity is possessed by 9 : 10-dihydroxy-9 : 10-di-*n*-propyl-9 : 10-dihydro-1 : 2 : 5 : 6-dibenzanthracene. Potency was also observed in the highly unsaturated aliphatic acid, clupanodonic acid, whilst a feeble but definite oestrogenic action was found to be possessed by calciferol. Since neither of these compounds contains the phenanthrene ring system, it was considered that this was not essential for oestrogenic properties. A further interesting observation was made in the partial activity of 1-keto-1 : 2 : 3 : 4 : 5 : 6 : 7 : 8-octahydroanthracene. This substance produced an advanced pro-oestrus, but full cornification could not be produced no matter how much of the material was administered.

A series of derivatives of acenaphthene was studied because these compounds contain a three-ring system arranged in a manner different from that in phenanthrene. These were tested on ovariectomized rats in the manner described in the above-mentioned publications. Table I shows the

Table I

Derivatives of acenaphthene	Method of preparation	Dose in mg.	% positive
1 : 2-Dihydroxy-1 : 2-di-*n*-propyl acenaphthene	Maxim (1929)	100	Nil
1 : 2-Dihydroxy-1 : 2-diphenyl acenaphthene	Beschke (1909)	100	Nil
1 : 2-Dihydroxy-1 : 2-dibenzyl acenaphthene	Maxim (1929)	100	Nil
1 : 2-Dihydroxy-1 : 2-di-α-naphthyl acenaphthene	—	100	100
		10	100
		1	20
1 : 1-Diphenyl acenaphthenone	Beschke (1909)	100	Nil
1 : 1-Di-α-naphthyl acenaphthenone	—	100	100

results obtained with this group of substances. It will be observed that the most potent member of the series is 1 : 2-dihydroxy-1 : 2-di-α-naphthyl acenaphthene. In doses of 10 mg. this compound produces prolonged oestrus. This new substance confirmed our assumption that the phenanthrene ring system was unnecessary for oestrogenic activity and led to experiments with a number of aromatic carbinols (Table II). This series of compounds showed surprising differences in behaviour. Thus, whilst diphenyl-α-naphthyl carbinol possesses full activity in doses of 100 mg. the corresponding diphenyl-β-naphthyl carbinol and triphenyl carbinol were both without action. The effect of hydroxy groups in the aromatic nucleus was then studied and several phenolic diphenyl methane derivatives were investigated with results as shown in Table III. 3 : 3′- and 4 : 4′-dihydroxy-diphenyl methane both showed activity, the latter much greater than the former, but the corresponding dicarboxylic acids were without effect. Many potent substances were found in derivatives of 4 : 4′-dihydroxy-diphenyl methane. From this series it appears that the alkyl substituents on the central carbon atom have no influence on the efficacy of the substance. A single phenyl nucleus has a distinct depressing action, and the diphenyl derivative is completely inert. The introduction of methyl groups in the 3 : 3′ positions appears to diminish the potency in some cases.

TABLE II

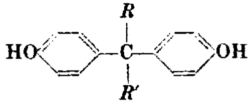
Substance	Method of preparation	Dose mg.	% positive
Diphenyl carbinol	—	100	Nil
Triphenyl carbinol	—	100	Nil
Phenyl-α-naphthyl carbinol	Acree (1904)	100	Nil
Di-α-naphthyl carbinol	Schmidlin and Massini (1909)	100	Nil
Diphenyl-α-naphthyl carbinol	Acree (1904)	100	100
Diphenyl-β-naphthyl carbinol	Ullmann and Mourawiew-Winigradoff (1905)	100	Nil
1 : 8-Di-α-naphthoyl naphthalene	—	100	Nil
Benzil	—	100	Nil
Hydrobenzoin	—	100	Nil
Phenyl hydrobenzoin	Acree (1904)	100	Nil
α-Naphthyl benzoin	Acree (1904)	100	60
α-Naphthyl hydrobenzoin	Acree (1904)	100	80
Benzpinacone	—	100	Nil
Benzpinacoline	—	100	Nil
9-Hydroxy-9-α-naphthyl fluorene	Ullmann and Wurstemberger (1905)	100	Nil

Dihydroxy benzophenone was less powerful than the corresponding diphenyl methane compound. It is interesting to note the surprising effect of reducing 4-hydroxy-triphenyl carbinol to 4-hydroxy-triphenyl methane,

TABLE III

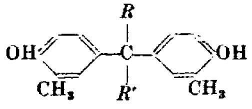
Derivatives of diphenyl methane:	Method of preparation	Dose (mg.)	% positive
4-Hydroxy-diphenyl methane	Clemmensen (1914)	100	Nil
3 : 3′-Dihydroxy-diphenyl methane	Auwers and Rietz (1907)	100	40
4 : 4′-Dihydroxy-diphenyl methane	Eberhardt and Welter (1894)	100	100
Diphenyl-methane-3 : 3′-dicarboxylic acid	Schöpf (1894)	100	Nil
Diphenyl-methane-4 : 4′-dicarboxylic acid	Schöpf (1894)	100	Nil
4 : 4′-Dihydroxy-diphenyl-methane-3 : 3′-dicarboxylic acid	Kahl (1906)	100	Nil
2 : 5-Dihydroxy-diphenyl-methane-carboxylic acid lactone	—	100	Nil
α-(2-Hydroxy-5-methyl-phenyl) phthalide	—	100	Nil
Benzilic acid	—	100	Nil
2 : 2′-Dihydroxy-di-α-naphthyl methane	Fries and Hübner (1906)	100	Nil
α : α-Diphenyl ethylene	Klages (1902)	100	Nil

Derivatives of 4 : 4′-dihydroxy diphenyl methane (Easson, Harrison, McSwiney and Pyman 1934):



R	*R′*			
Methyl	Methyl		100	100
Methyl	Ethyl		100	100
Methyl	*n*-Propyl		100	100
Ethyl	Ethyl		100	100
H	*n*-Hexyl		100	100
H	Phenyl	Russanow (1889)	100	100
			10	20
Methyl	Phenyl	—	100	60
Phenyl	Phenyl	—	100	Nil

Derivatives of 4 : 4′-dihydroxy-3 : 3′-dimethyl-diphenyl methane (Easson, Harrison, McSwiney and Pyman 1934):

R	*R′*			
Methyl	Methyl		100	100
Methyl	Ethyl		100	100
H	*n*-Hexyl		100	Nil
Methyl	Phenyl		100	Nil
Di-(4-hydroxyphenyl)-1 : 1-*cyclo*hexane			100	100
Di-(4-hydroxy-3-methyl phenyl)-1 : 1-*cyclo*hexane			100	100

Derivatives of benzophenone:

4-Hydroxy-benzophenone	Heller (1913)	100	Nil
4 : 4′-Dihydroxy-benzophenone	Baeyer and Burkhardt (1880)	100	80
2 : 3 : 4 : 4′-Tetrahydroxy-benzophenone	—	100	60
2 : 3 : 4 : 3′ : 4′ : 5′-Hexahydroxy-benzophenone	Bleuler and Perkin (1916)	100	20

Derivatives of triphenyl methane:

4-Hydroxy-triphenyl acetic acid	Bistrzycki and Novakowski (1901)	100	Nil
4-Hydroxy-triphenyl carbinol	Bistrzycki and Herbst (1901)	100	Nil
4-Hydroxy-triphenyl methane	Bistrzycki and Herbst (1902)	100	100
Phenolphthalein	—	100	Nil
2-Hydroxy-5-methoxy-tri-phenyl-methane carboxylic acid lactone	Easson, Harrison, McSwiney and Pyman (1934)	100	Nil
2 : 4-Dihydroxy-4′ : 4″-dimethoxy-tri-phenyl-methane carboxylic acid lactone	Easson, Harrison, McSwiney and Pyman (1934)	100	Nil
2 : 4-Dihydroxy-triphenyl-methane carboxylic acid lactone	Easson, Harrison, McSwiney and Pyman (1934)	100	80
4-Hydroxyphenyl-di-α-naphthyl methane	Schmidlin and Massini (1909)	100	100
		10	100
4-Hydroxy-tetraphenyl methane	Baeyer and Villiger (1902)	100	Nil

and finally the increased activity obtained by substitution of α-naphthyl for phenyl in this molecule.

Investigation of a group of substances in which the linkage between the two phenol nuclei was varied provided many powerful agents which are given in Table IV.

TABLE IV

	Method of preparation	Dose (mg.)	% positive
Derivatives of diphenyl:			
2-Hydroxy-diphenyl	—	100	Nil
3-Hydroxy-diphenyl	—	100	Nil
4-Hydroxy-diphenyl	Schlenk (1909)	100	Nil
2 : 2′-Dihydroxy-diphenyl	—	100	Nil
3 : 3′-Dihydroxy-diphenyl	Häussermann and Teichmann (1894)	100	Nil
4 : 4′-Dihydroxy-diphenyl	Hirsch (1889)	100	100
4 : 4′-Dihydroxy-3 : 3′-dimethyl diphenyl	Hobbs (1888)	100	Very slight activity
Diphenyl-4 : 4′-dicarboxylic acid	—	100	Nil
2-Hydroxy fluorene	Diels (1901)	100	Nil
4 : 4′-Dihydroxy-1 : 1′-dinaphthyl	—	100	Nil
Derivatives of α : β-diphenyl ethane:			
α-Hydroxy-diphenyl ethane	Schmidlin and Garcia-Banùs (1912)	100	Nil
2-Hydroxy-diphenyl ethane	Kostanecki, Rost, Szabrânski (1905)	100	Nil
4-Hydroxy-diphenyl ethane	Stoermer and Kippe (1903)	100	Nil
4-4′-Dihydroxy-diphenyl ethane	Heumann and Wiernik (1887)	100	100
Stilbene	Wislicenus and Endres (1903)	100	100
		25	100
2-Hydroxy stilbene	Kostanecki and Tambor (1909)	100	Nil
4-Hydroxy stilbene	Hewitt, Lewcock and Pope (1912)	100	100
		10	100
		5	40
3′-Hydroxy-stilbene-α-carboxylic acid	Werner (1895)	100	Nil
4 : 4′-Dihydroxy stilbene	Auwers (1903)	100	100
		10	100
		5	80
4 : 4′-Dimethoxy stilbene	Elbs (1893)	100	Nil
Tolane	Fittig and Ammand (1873)	100	Nil
4 : 4′-Dihydroxytolane	Zincke and Münch (1904)	10	100
		5	80
Triphenyl ethylene	Klages and Heilmann (1904)	10	100
		5	50
Tetra-phenyl ethylene	Norris, Thomas and Brown (1910)	100	Nil
Derivatives of α : γ-diphenyl propane:			
4-Hydroxy-phenyl-styryl ketone	Kostanecki and Tambor (1899)	100	Nil
Phenyl-4-hydroxy-styryl ketone	Bablich and Kostanecki (1896)	100	Nil
4 : 4′-Dihydroxy-diphenyl propane	—	100	100
2 : 4 : 6 : 4′-Tetrahydroxy-diphenyl propane	—	100	100
Phloretin	Schiff (1874)	100	100
Phloridzin	—	100	80
α : δ-Diphenyl butadiene	Thiele and Schleussner (1899)	25	100
4 : 4′-Dihydroxy-dibenzylidene acetone	Zincke and Muhlhausen (1903)	100	Nil
4 : 4′-Dihydroxy-α : ε-diphenyl pentane	Borsche (1919)	80	100

After the publication of the activity of diphenyl ethylene (Dodds and Lawson 1937*a*), Robson and Schönberg (1937) described the activity of triphenyl ethylene. The full series was described later (Dodds, Fitzgerald and Lawson 1937).

Only the 4 : 4′-dihydroxy derivative of diphenyl was found to have oestrogenic properties, which were not possessed by compounds having the hydroxy groups in other positions. Introduction of methyl groups in the adjacent position to the hydroxyl in this substance considerably decreased its activity. This was confirmed by Grant (1937) who found that 4 : 4′-dihydroxy diphenyl promoted mammary growth in young adult male guinea-pigs, while the 4 : 4′-dihydroxy-3 : 3′-dimethyl diphenyl was without effect. Dihydroxy-diphenyl ethane was found to possess full activity in doses of 100 mg. and the introduction of an ethylenic linkage between the α and β carbon atoms was accompanied by a great increase in potency, which further unsaturation appears not to affect. Thus 4 : 4′-dihydroxy tolane possesses considerable oestrogenic power in doses of 5 mg. It is interesting to note that the naturally occurring glucoside phloridzin possesses considerable activity whilst phloretin, obtained by hydrolysis of phloridzin, is more powerful. Increase in length of the connecting carbon chain appears not to affect the action of the substances since 2 : 4 : 6 : 4′-tetra-hydroxy-α : γ-diphenyl propane (from phloretin by reduction of the carbonyl group) and 4 : 4′-dihydroxy-α : ϵ-diphenyl pentane both gave positive results in doses of 100 mg.

The hydrocarbon stilbene itself possessed considerable activity which is increased by the introduction of a further phenyl nucleus (triphenyl ethylene), whilst tetra-phenyl ethylene is without action. In Table V is shown the effect of linking two molecules of phenol by oxygen or nitrogen. Full activity was shown by 4 : 4′-dihydroxy diphenyl ether in doses of 100 mg. and slightly less by the mono-hydroxy compound. Similar derivatives of diphenylamine were inert. The properties of stilbene and its

TABLE V

	Method of preparation	Dose mg.	% positive
Derivatives of diphenyl ether:			
Diphenyl ether	—	100	Nil
2-Hydroxy-diphenyl ether	Ullmann and Stein (1906)	100	Nil
4-Hydroxy-diphenyl ether	Häussermann and Bauer (1896)	100	80
2 : 2′-Dihydroxy-diphenyl ether	Ullmann and Stein (1906)	100	Nil
2 : 4′-Dihydroxy-diphenyl ether	—	100	Nil
4 : 4′-Dihydroxy-diphenyl ether	Häussermann (1897)	100	100
4-(*p*-Hydroxyphenoxy) benzoic acid	—	100	Nil
Thyronine	—	90	Nil
Derivatives of diphenylamine:			
4-Hydroxy diphenylamine	Schneider (1899)	100	Nil
4 : 4′-Dihydroxy diphenylamine	Knoevenagel (1914)	100	Nil

hydroxy derivatives led us to examine similarly constructed compounds containing only a single aromatic ring. *p*-Propenyl phenol induced full oestrus in doses of 100 mg. but the isomeric *p*-allyl phenol proved completely inactive as did also the hydrocarbon propenyl benzene. It is interesting to note that methylation or the presence of a methoxyl in the 2 position renders such feebly active substances inert, as anethole and *iso*-eugenol were both without effect. All the *p*-*n*-alkyl phenols from *p*-cresol to *p*-*n*-octyl phenol, and also *p*-*n*-hexadecyl phenol, were tested, but only the propyl derivative had oestrogenic properties. Other phenols and their derivatives are found in Table VI.

TABLE VI

Derivatives of phenol	Method of preparation	% positive with 100 mg. dose
2-Allyl phenol	—	Nil
4-Allyl phenol (chavicol)	—	Nil
4-Propenyl phenol (anol)	—	100
4-*tert*-Butyl phenol	—	Nil
2-*n*-Amyl phenol	—	Nil
4-*tert*-Amyl phenol	—	80
4-*n*-Amyl-2-methyl phenol	—	Nil
2-Methoxy-4-*n*-butyl phenol	—	Nil
2-Methoxy-4-allyl phenol (eugenol)	—	Nil
2-Methoxy-4-propenyl phenol (*iso*-eugenol)	—	Nil
4-*iso*-Propyl-3-methyl phenol	—	Nil
4-*n*-Hexyl resorcinol	—	Nil
4-*cyclo*-Pentyl phenol	—	Nil
4-*cyclo*-Hexyl phenol	Bartlett and Garland (1927)	80
3 : 4 : 5-Triphenyl phenol	Smith (1893)	Nil
Hydroquinone	—	Nil
4-Hydroxybenzaldehyde	—	Nil
4-Hydroxy benzoic acid	—	Nil
4-Hydroxy-phenyl acetic acid	—	Nil
4-Hydroxy-β-phenyl ethyl alcohol	v. Braun (1912)	30
4-Hydroxy hydrindene	Mills and Nixon (1930)	Nil
5-Hydroxy hydrindene	Mills and Nixon (1930)	Nil
5-Hydroxy-α-hydrindone	Auwers and Hilliger (1916)	Nil
7-Hydroxy-α-hydrindone	Auwers and Hilliger (1916)	Nil

It would appear from a study of this table that the most interesting compounds are those with the 3-carbon atom chain para to the hydroxyl group. Thus both *p*-hydroxy-propyl benzene and *p*-hydroxy-propenyl benzene are active whilst *p*-hydroxy-allyl benzene is inactive. A very complex situation was encountered with certain specimens of *p*-hydroxy-propenyl benzene or anol. This compound was made by demethylation of anethole with strong alkali and alcohol. Certain specimens were found to possess a very high degree of activity (Dodds and Lawson 1937*a*), and later it was shown that this activity was due to contamination with some substance, possibly a polymer of anol. It was shown that substances of high activity could always be detected in the mother liquor obtained from crystallizing anol from ether or chloroform (Dodds and Lawson 1937*b*). From these experiments it is obvious that either a polymeride or derivative of anol must possess very great activity. In this connection it is interesting to note that Serini and Steinruck (1937) have obtained highly active crystalline substances as by-products in the demethylation of anethole.

PREPARATION OF MATERIALS

Most of the substances were prepared by methods already described, to which references are given in the tables.

4-*cyclo*-Pentyl phenol, which is described by v. Braun (1929), is more conveniently prepared from *cyclo*-pentyl bromide and phenol in the presence of zinc chloride by the method of Bartlett and Garland (1927) for the *cyclo*-hexyl derivative.

The following compounds are new:

1 : 2-*Di-hydroxy*-1 : 2-*di-α-naphthyl acenaphthene*. To an ice-cold Grignard solution (4 mols) from α-bromonaphthalene, was added in portions, with stirring, finely powdered acenaphthene quinone (1 mol). After 1 hr. the mixture was heated on the water bath for 4 hr. after which the whole was decomposed with ice and ammonium chloride. The ethereal solution was dried over sodium sulphate, the ether removed and the resinous residue dissolved in 10 parts of benzene from which it crystallized slowly, giving an almost pure product which on recrystallization from benzene gave a colourless, microcrystalline powder, m.p. 142°.

(Found, after air drying: C, 88·1, 88·6; H, 5·4, 5·5; loss when dried in a vacuum at 90°, 15·0 %. $C_{32}H_{22}O_2$, C_6H_6 requires C, 88·35; H, 5·45; C_6H_6, 15·1 %.)

1 : 1-*Di-α-naphthyl acenaphthenone*. To a solution of the di-α-naphthyl-acenaphthene diol (5 g.) in acetic acid (50 c.c.) at the boiling-point, concentrated hydrochloric acid was added drop by drop. The solution became bright yellow and crystals appeared. After cooling, these were filtered off and washed with alcohol. The substance is sparingly soluble in organic solvents and crystallised from 100 parts of xylene in pale yellow needles, m.p. 289°. (Found: C, 91·1; H, 4·9. $C_{32}H_{20}O$ requires C, 91·4; H, 4·8 %.)

1 : 8-*Di-α-naphthoyl naphthalene.* A solution of di-α-naphthyl acenaphthene diol (3 g.), chromic anhydride (0·75 g.) in acetic acid (30 c.c.) was boiled for 5 min. Water was then added till a turbidity appeared and the solution was cooled. The crystals were separated and washed with alcohol. 1 : 8-Di-α-naphthoyl naphthalene crystallized from toluene in prisms, m.p. 227–228°. (Found: C, 88·1; H, 4·8. $C_{32}H_{20}O_2$ requires C, 88·0; H, 4·6 %.)

2 : 4 : 6 : 4′-*Tetra-hydroxy-α-γ-diphenyl propane.* Phloretin (3 g.) was boiled under reflux with amalgamated zinc wool (10 g.) and hydrochloric acid (15 c.c. diluted with 15 c.c. water) for 18 hours. On cooling the solution gave crystals which after two recrystallizations from water melted at 158–159° and contained one molecule of water of crystallization. (Found: C, 64·9; H, 6·6. $C_{15}H_{16}O_4$, H_2O requires C, 64·7; H, 6·5 %.)

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SUMMARY

1. The phenanthrene ring system is not necessary for oestrogenic activity.

2. Substances containing two phenol groups joined by a carbon chain are active. The number of carbon atoms, the position of double bonds and of substituent groups attached to the carbon chain all vary the activity.

3. Substituents in the aromatic nucleus apart from the hydroxyl group appear to lessen activity.

4. It is possible to obtain activity in compounds containing only one benzene ring. Thus both para-hydroxy *n*-propyl and propenyl benzene are active. The corresponding allyl compound is without activity. The propenyl compound (anol) readily undergoes polymerization under certain conditions to form very highly active substances.

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The development *in vitro* of the mammalian gonad. Ovary and ovogenesis*

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[Plates 9–11]

INTRODUCTION

Several investigators have tried to cultivate *in vitro* the mammalian and avian gonad, usually with the object of obtaining the growth and differentiation of the germ cells under *in vitro* conditions. The results so far obtained, however, have been disappointing. Champy (1920) is the only

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author, so far as we know, who has definitely claimed to observe some differentiation of the germ cells *in vitro*. He states that in testicular explants from the mature rabbit he observed the transformation of spermatogonia into spermatocytes, but the process did not advance beyond the leptotene phase of meiosis. Champy's findings, however, have not been confirmed by other investigators, probably owing to the difficulty of cultivating mature testicular tissue.

In birds, progressive differentiation of the germ cells *in vitro* has not been recorded. Cultures of ovarian and testicular tissue of the embryonic fowl have been studied by Fano and Garofolini (1927 and 1928). The explants formed an epithelial outgrowth which was soon replaced by what appeared to be fibroblasts. Dantchakoff (1932) cultivated the undifferentiated gonad of 3½-day fowl embryos and described three types of cells in her cultures: (*a*) large amoeboid cells—Waldeyer's "Ureier" or "Urkeimzellen"—which *in vitro* developed pseudopodia and showed active amoeboid movement, (*b*) connective tissue strands, (*c*) epithelium-like tissue. Wermel (1933) made cultures of the undifferentiated gonad and of embryonic and adult testes and observed growth of both the somatic and germinal tissue. Muratori (1935 and 1937) cultivated gonad tissue from embryonic and newly hatched fowls, but although he describes the nuclear pattern of the germ cells emigrating from the explants, he makes no mention of any differentiation occurring in the explanted gonocytes.

Several workers have grown mammalian gonad tissue *in vitro*.

Maccabruni (1913) studied cultures of the human foetal ovary and observed proliferation from the explant of connective tissue only. Wolff and Zondek (1925) obtained proliferation of both connective tissue and epithelium from explants of human foetal ovaries and from the ovary of a 45-year old woman. Olivo (1934) obtained successful cultures of the cumulus oophorus tissue containing the egg from a human embryo, but failed to cultivate the granulosa tissue without the egg, from the same ovary. He also noted that the egg enlarged somewhat during cultivation and two large nuclei appeared in it.

Mjassojedoff (1925) found that the follicular cells of the rabbit ovary when growing *in vitro*, quickly lose their epithelial character and in 6–8 days become very like fibroblasts. Champy (1920, 1926, 1927) and Champy and Morita (1928) explanted ovarian and testicular tissue from mammals (rabbit, rat) and from some of the lower vertebrates, and observed that the germinal tissue proliferated *in vitro* in an undifferentiated form. Champy regarded this observation as evidence in support of his dedifferentiation theory. As stated above, Champy also described one stage of spermatogenesis *in vitro* which took place in a 9-day culture of adult rabbit testis after all the spermatocytes present at the time of explantation had degenerated. Esaki (1928) studied the fate of interstitial and Sertoli cells in explants of rabbit and guinea-pig testis. He stated that the interstitial cells are derived from the mesenchyme and that *in vitro* they become transformed into spindle-shaped elements indistinguishable from ordinary fibroblasts; the Sertoli cells became rounded and phagocytic in culture. Mihailoff (1937) recorded the survival *in vitro*, for about 70 days, of the seminiferous tubules of the immature rabbit. A few primitive germ cells (gonocytes) persisted in a more or less normal state during this period, but failed to differentiate. Both mesenchyme derivative and Sertoli cells grew and migrated actively.

The object of the present investigation was to cultivate the entire ovary of embryonic and new-born rats and mice and to study its behaviour *in vitro* with special reference to the growth and differentiation of the germ cells. It was clear that if the germinal tissue could be made to differentiate *in vitro*, it would provide a new method for analysing the complex factors responsible for the growth and development of the ovary (Martinovitch 1937).

Of recent years something has been learned of the influence of the gonadotropic hormones on the normal functions of the ovary. Little is known, however, of the possible influence of the organ as a whole on the germinal tissue which it contains or of the inherent capacity of the germ cells for independent growth and differentiation. The results of the present experiments shed some light on both these problems and also on the vexed question of the length of survival of the mammalian ovum.

MATERIAL AND TECHNIQUE

Forty ovaries from rats and mice ranging in age from the 16th day of embryonic life to the 4th day after birth were cultivated *in vitro* by the watch-glass method (Fell and Robison 1929). The culture medium was composed of equal parts of chicken embryo extract prepared in Pannett and Compton's solution and chicken plasma. In some experiments 1% glucose was added to the medium. One ovary from each animal was explanted *in vitro* and the corresponding ovary was fixed as a control. In order to compare the rate of growth of the ovum *in vitro* and *in vivo*, the normal ovaries of 8-, 5- and 2-day-old rats were also fixed and sectioned.

The explants and some of the controls were fixed in Allen's modification of Bouin's fluid and serially sectioned. The remaining controls were fixed in unmodified Bouin's fluid. Sections were stained with Meyer's haematoxylin, Heidenhain's haematoxylin or by Feulgen's method counterstained with light green.

Methods of preparation of the ovaries employed in this work were not of the nature to reveal any of the various cytoplasmic constituents.

OBSERVATIONS

The results showed that the entire process of oogenesis can take place *in vitro* under appropriate conditions of cultivation. Oogenesis *in vitro* does not differ materially from the normal process *in vivo*, and the series of development of explanted ova are therefore described in chronological order, beginning with a stage of development just preceding the appearance of the first meiotic figures. For the sake of convenience, the rat ovaries only are considered, although the mouse ovaries behave in a similar way.

The development in vitro *of ovaries from* 15–16*-day embryonic rats.*

Normal (*control*) 15–16*-day embryonic ovary* (fig. 1, Plate 9)

This ovary was taken from one of the same batch of embryos as the explanted ovaries B XI_1 to B XI_6 inclusive, and was fixed at the beginning of the culture period as a control.

The dense mass of tissue composing the ovary contains two main types of cell: (*a*) the oogonia, large cells with big spherical nuclei and (*b*) the somatic ovarian cells which are smaller and contain round or oval nuclei. The oogonia are most abundant towards the centre of the ovary and are very scarce in the ovarian epithelium.

In some regions of the ovary there is no visible sign of a developing albuginea, the germinal epithelium being continuous with the underlying tissue, but elsewhere, especially near the site of attachment to the body wall, some of the mesenchyme cells immediately beneath the epithelium have begun to elongate. Similar elongated mesenchyme cells occur in the interior of the ovary where they appear in section as single rows indicating the boundaries of the future germinal nests and cords.

The nuclei of the oogonia contain numerous very fine, dust-like particles of chromatin and several larger, usually peripheral, knots of intensely staining chromatin. Although the particles seem to be connected by very fine protoplasmic bridges, no sign of that formation of chromatin threads which heralds the onset of meiosis, could be detected. In general appearance the oogonia resemble the spermatogonia—"gonies poussiéreuses" of Regaud—which occur in the testis of a new-born rat, except that the fine particles of chromatin are slightly coarser and the chromatin knots less distinct than in the spermatogonia. Many germ cells are undergoing mitotic division, metaphase being much the most numerous stage. The cells of the mesenchyme and of the germinal epithelium are also dividing actively. A few germ cells are degenerating.

The ovary contains many nucleated red blood cells which in the explanted organs sometimes persisted for as long as 10 or 12 days.

Explanted ovary after 19 *hr.* in vitro (explant No. B XI_1)

The most conspicuous change in the general structure of the ovary, as compared with that of the control described above, is the increase in the number of spindle-shaped cells which now form well-defined parallel single cell rows running in a dorso-ventral direction with regard to the gonad's original attachment to the body wall. The future nests and cords are thus more clearly outlined. The mesenchyme cells beneath the germinal epithelium, though not yet spindle-shaped, are more numerous, but a typical stroma has not so far differentiated in this region.

The cells of the germinal epithelium are irregular in shape and arrangement, some being only loosely attached to the surface of the explant.

The injured surface of the organ is covered by a single, discontinuous layer of epithelial cells. Many white blood cells adhere to the ovarian wall, but there is no migration of the ovarian tissue into the medium.

The germ cells, which are very abundant, differ from those of the control. The powdery particles of chromatin have increased both in number and staining intensity, and the large chromatin knots have either partially or completely disappeared. In a few of the germ cells the chromatin granules have a linear arrangement which foreshadows the thread-like formations of the leptotene phase.

The number of degenerate germ cells is about the same as in the control. No central necrotic area ever forms in cultures of 15–16-day ovaries.

Explanted ovary after 42 *hr.* in vitro (explant No. B XI_2)

The first stages of the meiotic prophase—leptotene and synaptene—appear at this period of cultivation. The leptotene phase is represented by numerous fine threads either scattered evenly through the nucleus (fig. 2, Plate 9) or slightly polarized (fig. 3, Plate 9). Cells at a stage intermediate between the preceding "dusty" stage and the leptotene are still fairly common.

Synaptene figures are already numerous. The chromatin threads have begun to contract towards one end of the nucleus. A double structure was not observed before the contraction stage, although some of the threads were distinctly looped forming the familiar bouquet figure. In later stages of contraction, the closely packed threads appear as an almost uniform mass of chromatin to one side of the otherwise clear nucleus, with merely a few loops protruding at the margin to indicate the filamentous nature of the mass.

The number of dividing oogonia, mostly in metaphase, is still very great (fig. 4, Plate 9).

The degenerating germ cells appear as an intensely stained, ellipsoid mass of nuclear material capped with a deeply staining crescent of cytoplasm.

The connective tissue of the stroma has not differentiated much further, although in the region of the hilum a narrow band of elongated cells clearly demarcates the ovarian tissue proper from a tiny strip of mesovarian tissue included in the explant. The germinal epithelium consists of a single layer of flattened cells, separated from the underlying germ cells by a sheet of rather flattened connective tissue cells which represent the developing albuginea. Ova do not occur either in the germinal epithelium or in the albuginea. Cells have now begun to migrate from the surface of the ovary.

Explanted ovary after 50 *hr.* in vitro (explant No. B XI_3)

Structures resembling Pflüger's cords make their first appearance at this stage of cultivation (fig. 5, Plate 9). The mesenchyme separating groups of germ cells has become compressed and flattened to form well-marked partitions, thus producing a cord-like structure in the ovarian tissue. The cords are packed with oocytes and Sertoli cells, the former being the more numerous. Only a few cells of the oogonial type are present, the proportion of oocytes having greatly increased. Leptotene and synaptene figures are about equally represented. The healthy and normal appearance of the explanted ovary at this stage is striking.

Fewer germ cells are dividing than before, and the mitotic wave has receded towards the periphery of the organ. It is noteworthy that some of the metaphase figures are abnormal; the chromosomes are clumped together in certain germ cells, and in others spherical pycnotic masses of chromatin of different sizes are scattered throughout the cell body. It is most unlikely that such cells complete division. On the other hand, mitotic figures in the somatic cells, both in the germinal epithelium and in the cords, appear normal (fig. 6, Plate 9) (see Rauh 1926).

The single-layered germinal epithelium contains an unusually large number of dividing cells. In this explant there is no true albuginea, and the nests of germ cells lie close to the surface.

A few cells of the germinal epithelium and underlying mesenchyme have emigrated from the organ. In all cultures of 15–16-day embryonic ovaries the zone of outgrowth was found to be small, although its area could be increased by adding glucose to the medium and was of the familiar fibroblastic type. In explants from embryos of this age the emigration of germ cells was never, and the formation of epithelial sheets only occasionally, observed in the zone of growths, though it should be emphasized that no special study was made of the nature of the outgrowth in these cultures.

Explanted ovary after 71 *hr.* in vitro (explant No. B XI_4)

At this stage the vast majority of the oocytes are at the advanced contraction phase of meiosis (synizesis) (fig. 7, Plate 10). In preparations stained by Feulgen's method, the chromatin appears as a deeply stained, almost impenetrable mass, but in those stained with Meyer's haematoxylin the individual threads are sometimes clearly visible. The leptotene nuclei are distributed along a narrow zone parallel to the surface of the organ, and intermingled with them are the dividing oogonia, now greatly reduced in number.

The strands and partitions of connective tissue between the nests of germ cells have increased in both number and size, and the germinal nests have become correspondingly smaller and more numerous. There is no appreciable change in the germinal epithelium or underlying stroma.

Explanted ovary after 96 *hr.* in vitro (explant No. B XI_5)

At this stage many of the oocytes have emerged from the contraction phase and the chromatin threads have reappeared. The oocytes appear on the whole less healthy than in the preceding culture, as indicated by the variations in the appearance, number and distribution of the chromatin threads and in the size of the nuclei. In some cells the threads are long and fine and in others short and stubby; sometimes they appear as a continuous, highly convoluted spireme, filling the whole nucleus, but in others they are definitely polarized, the spireme-like arrangement occurs in large nuclei only, but otherwise there seems to be no correlation between the appearance of the chromatin threads and the size or shape of the nucleus containing them.

It is often difficult to tell which of the various forms of chromatic thread represent successive stages in a continuous process, and which are mere abnormalities. It is also impossible to determine which of the abnormalities represent an irreparable injury to the cell, and which are merely reactions to new conditions of life and not necessarily lethal.

Many of the germ cells are obviously necrotic and oocytes are degenerating at every stage of development; very few are in mitosis and these appear abnormal. The larger nests of germ cells are breaking up into smaller groups.

The germinal epithelium is no longer a continuous layer, owing to the continual migration of cells into the surrounding medium and to the failure of the remaining cells to divide fast enough to fill the gaps thus produced. The underlying mesenchyme cells, many of which are in mitosis, have become definitely flattened.

A conical mass of connective tissue has begun to differentiate near the original hilum of the ovary, the base of this cone coincides with the former area of attachment and the apex is thrust into the centre of the ovary.

Explanted ovary after 6–6·16 *days* in vitro (explants No. B XI_6 and B $XII_{1,2}$)

Nearly all the germ cells have now evolved from the contraction phase. The chromatin filaments, once more distinct, now form the thick, short, polarized loops characteristic of the post-contraction "bouquet" phase of the normal embryonic ovary (fig. 8, Plate 10). The oocytes, though greatly reduced in number, look healthier than in the ovary of the preceding stage. Some diplotene nuclei have already appeared.

The germinal epithelium has almost disappeared and is represented by a few scattered epithelial cells clinging to the surface of the ovary.

After the virtual disappearance of the germinal epithelium, the surface of the ovary is covered by the albuginea, which is composed of three or four layers of flattened fibrocytes. There is a striking difference in appearance between the typical connective tissue cells of the albuginea and the mesenchyme-like cells of the deeper layers of the ovary.

Up to this time the size of the ovary has remained unaltered.

Explanted ovary after 6·21–8 *days* in vitro (explants Nos. B $XII_{3,4,5,6}$)

In vitro the pachytene phase of meiosis is brief. As stated above, a few diplotene nuclei had already appeared at the preceding stage, although most of the oocytes were in the pachytene phase.

Diplotene nuclei become much more numerous by about the 7th day of incubation (fig. 9, Plate 11). Many nuclei contain discontinuous, double threads which have been interpreted as representing the homologous chromosomes separating after their intimate contact during the zygotene phase. Such nuclei were most numerous in a 7·2-day culture, but in sister cultures obtained from the same litter and fixed a few hours before and after 7·2 days they were comparatively rare. This indicates that the separation of the homologous chromosomes occurs fairly quickly. It is interesting that in many of the oocytes which have passed through the pachytene phase, the chromatin loops much resemble a fine spireme. These loops are not so fine as the "smooth" leptotene threads and have a beaded appearance. In Feulgen preparations the nucleoli of the germ cells are faintly stained with light green.

The explants on the whole seemed quite healthy, although many oocytes were degenerating. The general structure was the same as in the preceding stage.

Although no accurate timing of the duration *in vitro* of *synaptene stage* has been attempted in these experiments, it has been found that on the whole it agrees fairly well with that recorded by Pratt and Long (1917) in normal ovaries.

Later development of the explanted ovary up to the 30th day in vitro

The stages in the development of the explanted ovary subsequent to the diplotene phase may be described together.

In normal development, according to the usual view, the diplotene phase is followed by a resting stage in which the individuality of the chromatin threads seems to disappear completely. The nucleus then contains an achromatic network in which are scattered many small chromatin granules and from one to three larger chromatin nucleoli. Most of the oöcytes remain unchanged in this condition for weeks, months or, according to some authors, even for years, but a few enlarge and eventually become full-grown ova.

Somewhat similar changes occur *in vitro*. After the diplotene phase the nuclear material becomes less chromophil and breaks down into one to three chromatin nucleoli and a number of smaller particles. Interconnecting achromatic strands are often extremely hard to see. The chromatin granules are coarser and less deeply staining than those of the normal oocyte. Sometimes the nuclear material consists of a nucleolus and a loosely connected, amorphous mass of particles with no apparent organization. The resting ova change little during subsequent cultivation.

As in the normal ovary, some of the resting ova in the explants begin to enlarge. Enlargement of the cytoplasm is accompanied by growth of the nucleus and increase of the chromatin. In the full-grown ova, the chromatin particles are large and coarse, and one or two large, spherical plasmasomes are present. The plasmasomes differ from the normal in being "less chromophil" and more clearly visible, a difference which is due to the fact that in the explants the surface of the plasmasomes is often free from the adherent particles of chromatin by which they are observed in the normal ovum. In nearly full-grown ova the nucleus assumes an excentric position. The ova are usually spherical and, unlike the normal ovum, appear completely devoid of a zona pellucida.

Abnormally shaped ova sometimes occur. Fig. 10 (Plate 11) shows one that is ring-shaped in section, the interior being occupied by a large, hollow space. A "peculiarity" of all ova cultivated *in vitro* is that even in the best fixed preparations they appear shrunken, so that a clear, ring-like space, sometimes bridged by fine radially distributed strands of protoplasm, intervenes between the ovum and the surrounding tissue. Whether this appearance is an artifact or whether it also occurs in the living explant has not been ascertained.

Some of the ova degenerate. Large ova do so by a process of karyorrhexis. The chromatin material breaks down into deeply stained fragments of various sizes, which are at first irregular in outline but gradually become spherical.

No normal maturation division has been observed in the older cultures, but one abnormal figure was found in a 25-day culture. This dividing ovum has passed *in vitro* through all the stages of differentiation and growth from an oogonium to a maturing ovum.

From the foregoing description it is seen that the development of the explanted ovary during synapsis differs little from that of the normal organ. After this stage, however, it shows a divergence from the normal which may be considered as peculiar to conditions *in vitro*. Two chief abnormalities occur in culture: (1) partial or total failure of the Sertoli cells to develop into Graafian follicles; (2) failure of the growing ovum to move towards the interior of the ovary. As a result of these abnormalities, nearly full-grown ova, either naked or surrounded by a few follicular cells, are clustered together near the surface of the ovary in what are really the original germinal nests and cords (fig. 11, Plate 10).

As *in vivo* only a small number of oocytes at a time begin to grow, the vast majority remaining unchanged. *In vitro*, the first batch of growing ova attain their maximum size 18 days after explantation, a period corresponding to 13 days of extra-uterine life. Measurements show that the largest size reached in culture is a diameter of 50–55 μ. This first batch of ova soon degenerates and is replaced by others. After 18 days' cultivation, ova of almost all sizes are to be found in the same ovary. Growth of the ovum *in vitro* is somewhat retarded as compared with the normal.

The explanted ovary does not enlarge very much, and after a month's cultivation is only about double its original volume. It enlarges in three ways: (1) by the growth of the ova, (2) by the formation of a primary follicle around certain oocytes, (3) by multiplication of the stroma cells. Many connective tissue cells are lost by migration into the medium and by degeneration.

The ovarian stroma undergoes very slight differentiation *in vitro* and the cells retain their primitive, mesenchymal appearance even after several weeks' cultivation. In the centre of the explant some typical stroma appears consisting of fairly dense fibrous tissue and a few scattered cells but elsewhere the intercellular fibres are very scanty. Medullary cords never develop in the explanted mouse ovary.

The germinal epithelium, as stated above, usually disappears after a week's cultivation, partly owing to migration of cells into the medium and partly owing to loss through mechanical damage inflicted during subcultivation. Sometimes, however, a few clustered cells of epithelial appearance cling to the surface of the explant for several weeks.

As already mentioned, no central area of necrosis appears in explanted ovaries from 16-day embryos, but degeneration changes affecting the entire organ begin to occur after 26–28 days *in vitro*. The translucency of the healthy explant gradually disappears and the organ becomes opaque. After 30 days' cultivation the whole organ is necrotic.

The development in vitro *of ovaries from late embryonic and new-born rats*

It seemed interesting to compare the development *in vitro* of the ovaries from 16-day embryos with that of ovaries from older embryos and newly born rats. Accordingly ovaries from 17-, 18-, 19- and 20-day embryos, new born and 4-day rats were cultivated *in vitro*.

It was found that on the whole the more highly developed the ovary at the time of explantation, the further it will differentiate during subsequent cultivation. This is particularly true of the ovarian follicle but only up to a certain stage in its development. Thus an explanted ovary from a 19–20-day embryonic rat after 3 weeks' cultivation may contain numerous, small Graafian follicles which do not differ essentially from follicles of the same size in the normal ovary (cf. figs. 12 and 13, Plate 11). Moreover, these follicles have moved towards the centre of the explant, as *in vivo*, instead of retaining their original superficial position as in explants of the 16-day embryonic ovary (cf. figs. 11 and 12). The amount and arrangement of the stroma also approaches more closely that of the mature gland than does that of explants of younger embryonic ovaries.

Another difference between the older and younger ovaries in culture is the appearance in the former of a central necrotic area. This degenerate region is seen shortly after explantation but disappears a few days later, the dead matter being either resorbed or cast out. The formation of the central necrotic area may be due to poor aeration or to a slow exchange of metabolites or to both these factors. Its disappearance seems to show that after an interval the ovary adjusts itself to the conditions *in vitro*. A necrotic area may disappear completely, to reappear a second time and again vanish. Many of the residual ova survive *in vitro* until the entire ovary degenerates (fig. 14, Plate 11).

In the explanted ovary of a 4-day mouse, a typical maturation-division figure was observed after 9 days' cultivation (fig. 15, Plate 11). The appearance of the figure was preceded by an actual growth of the ovum, since measurements showed that in the control ovary the diameter of the largest ovum was $45\,\mu$, while the diameter of the dividing ovum in the culture was $52\,\mu$.

The growth and differentiation of the ova is never seriously disturbed by cutting off the blood supply, and nearly all the young ova, at whatever stage in the early meiotic prophase they are explanted, continue their normal development *in vitro*. There seems to be no phase of meiosis which is particularly susceptible to injury by the process of explantation; but it is doubtful whether the different phases are of equal resistance to prolonged cultivation.

DISCUSSION

Phenomena of meiosis and growth of the ovum

The results recorded in this communication show that the primitive germ cells can undergo *in vitro* all the normal changes of differentiation and growth up to the stage of the nearly full-grown ovum. The question arises as to how far the growth and development of the ovum is controlled by factors intrinsic in the cell and how far it is influenced by external agents.

Two kinds of external agent must be considered: (*a*) those supplied by the body as a whole, and (*b*) those supplied by the ovary. The distinction between the two classes is arbitrary, however, as interaction between all the factors concerned, both extrinsic and intrinsic, may play an important part in the development of the germ cells.

Explantation *in vitro*, while depriving the ovary of its natural environment, by no means removes it completely from the influence of the body. The two constituents of the culture medium, plasma and embryo extract, might contain agents produced by the soma, which acted upon the germ cells.

The somatic agents known to influence the differentiation of the gonad are: (1) gonadotropic hormones, and (2) sex hormones.

With regard to the possible influence of gonadotropic hormones in the medium on the development of the explanted ovary, Pincus (1936) quotes extensive experimental evidence showing fairly conclusively that the Graafian follicles can only be activated by these hormones after attaining a certain stage of maturity and that growth of the ovum and of the early follicle are independent of the hypophysis. Even if the gonadotropic hormones do stimulate oogenesis under normal conditions, however, it is unlikely that the plasma and embryo extract contain enough of these substances to influence the explanted ova (see Robson 1934). On these grounds, therefore, it seems fairly safe to exclude the gonadotropic hormones as possible somatic agents stimulating oogenesis in the explants.

Whether the sex hormones in the culture medium influence oogenesis *in vitro* and if so, in what way, is difficult to determine. The recent experiments of Dantchakoff (1936) and Willier, Gallagher and Koch (1937) on the embryonic fowl, show that small doses of oestrin cause the histologically undifferentiated gonad of a zygotically determined male embryo to develop into an ovary and the genetically determined male germ cells, that it contains, to be transformed into female germ cells; whereas the male sex hormone causes the undifferentiated female gonad to become an ovotestis. But these authors make no clear statement whether the sex hormones may influence the process of gametogenesis under the conditions of their experiments. It should be emphasized, also, that these results refer to the avian gonad only, and whether they also apply to mammalian development is at present unknown.

The part that may, possibly, be played by the ovarian soma in inducing ovogenesis has received very little attention, and nothing is known of the possible interrelationship between the germ and other ovarian cells.

That the germinal epithelium is not essential to the growth of the ovum after *synapsis*, is proved by the fact that the epithelium usually disappears after a week's cultivation while the ova may continue to grow for another 20 days. The tissue culture experiments afford no evidence as to whether the stroma plays any specific part in the development of the ova, as this tissue persists, though in an undifferentiated form, throughout the culture period. They also give no information about the epithelial cells of the cortex in relation to oogenesis.

Very little is known of the ovum-follicle relationship in the early stages of the normal development of the ovum. In the explants nearly full-grown ova, 50–55 μ in diameter, developed completely devoid of follicle cells. This showed (*a*) that the early growth of the ovum is not dependent on the follicular epithelium, and (*b*) that in culture, growth of the follicle is not conditioned by growth of the ovum. Whether the ovum can complete its growth in the absence of a follicle is doubtful. The only full-sized ovum found in the explants, a binucleate egg 68 μ in diameter, occurred in an ovary from a 19–20 day embryo rat and was covered by a follicular envelope several cell layers thick. Why some growing ova should develop a follicle and others fail to do so is quite obscure. It was noted that ovaries explanted at birth formed more and larger follicles than those removed from embryos, suggesting that the capacity for follicular development increases with age.

With regard to the influence of the soma on oogenesis, the explantation experiments seem to indicate that in normal development the organism as a whole merely nourishes the germ cells and has no specific effect on their differentiation. What influence, if any, the ovary itself exerts upon the germ cells remains obscure, because although the explanted ovary develops a rather simplified structure *in vitro*, it is still too complex to allow of any definite conclusions being drawn.

Survival in vitro *of the ovum and the problem of neo-formation of the germ cells*

Whether the primitive germ cells persist, stored in the ovary, throughout post-embryonic life or whether they are continuously (Kingery 1917; Arai 1920; Butcher 1927, 1932; Hargitt 1930 *a*) or periodically (Allen 1923) renewed, are controversial questions.

The average life of the ovary *in vitro* is about one month, and in some explants many, and in others, a few, residual ova persist until the end of this period. The residual ova succumb only when the entire organ degenerates. In the explants, new ova are formed only by the mitotic division of pre-existing oogonia and not by neo-formation from the germinal epithelium. In the explanted ovaries of late embryos, but never in those of 15–16 days' gestation, a few ova occur in the germinal epithelium but they are at the same stage of development as those in the deeper parts of the ovary, and there is no evidence that such cells differentiate from the germinal epithelium. The germinal epithelium is usually completely lost after a week's cultivation and is thus finally eliminated as a possible source of new germ cells during the last three weeks of the culture period. All the germ cells in older cultures are embryonic in origin, i.e. they are cells which have passed through the early phases of meiosis. But even though tissue culture experiments show that neo-formation of germ cells from the germinal epithelium does not take place *in vitro*, they do not disprove the possibility that such proliferation might occur in normal post-embryonic life.

With the exception of Firket (1920), who claims that the primary germ cells of the albino rat degenerate 15 days after birth, those who support the view that the embryonic ova degenerate early agree that they normally survive for a period which exceeds the maximum culture period in the present experiments. In order to test the storage theory more completely it would be highly desirable, therefore, if a method was found of keeping the resting ova *in vitro* for longer periods of time than it has been possible heretofore.

On the other hand, these experiments destroy one of the arguments used by Hargitt and others against the storage theory. Hargitt (1930*a*) argues that "if an assumption be made that many latent primary follicles are stored in the mammalian ovary, a serious difficulty is encountered in explaining the stimulation of a few to renewed development, while others remain quiescent. Likely enough, some hormone might stimulate renewed activity, but why of a few not all of the same age?" In the tissue culture experiments the ovaries were explanted before the ova had begun to enlarge and, from the evidence given above, the explants throughout the culture period, were known to contain embryonic ova only. Nevertheless, as in the normal post-embryonic organ, only a few ova enlarged at any one time and many residual ova persisted unchanged until the end of cultivation.

That only a few out of a large number of apparently similar oocytes should enlarge at one time, is indeed a surprising fact for which no adequate explanation is at present forthcoming. The factors which cause the growth of any given ovum must be sought in the ovary itself. The position of the ovum is not concerned, since in the explants nearly full-grown ova often occur at the periphery of the organ (fig. 11, Plate 10) while on the other hand *resting* ova have been observed in the centre of a mouse ovary which had recovered from permanent ligation (unpublished data). Nor does the size of the ovary seem to be important in this connexion, since the volume of the explants is only about one-tenth that of the normal ovary of corresponding age.

I wish to express my thanks to Dr H. B. Fell, Director of the Strangeways Laboratory, for her help and advice throughout the investigation. I am also indebted to Mr V. C. Norfield, chief assistant at the laboratory, who was responsible for the photomicrographs.

Summary of results

1. The ovaries of embryonic and new-born rats and mice when explanted *in vitro* continue to live and develop for about 4 weeks.

2. Growth and differentiation of the germ cells occur in 100 % of cultures.

3. In explants of 16-day embryonic ovaries the oogonia undergo all but the final stages of normal differentiation and growth, and form nearly full-sized ova 50–55 μ in diameter. The ova in explants obtained from 2–5-day rats reach full-size and may undergo apparently normal maturation division.

4. The oocytes in the cultures only enlarge if they pass through the synaptic phases of meiosis.

5. Growth of the follicle in the explants is partly, and sometimes completely, suppressed.

6. The egg may enlarge in the absence of a follicular envelope.

7. The formation of a zona pellucida was never observed in ova growing *in vitro*.

8. Growth of the ovum is somewhat retarded *in vitro*.

9. Oocytes in culture multiply by the mitotic division of already differentiated oogonia and not by neo-formation from the germinal epithelium.

10. The germinal epithelium usually persists for about a week and then disappears owing to the emigration of its component cells into the surrounding culture medium.

11. Emigration of germ cells into the medium was not observed.

12. After 4 weeks' cultivation the embryonic rat ovary has about doubled its volume.

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DESCRIPTION OF PLATES

All figures are photographs of stained preparations

Plate 9

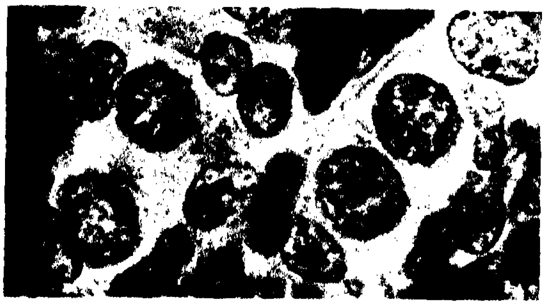
FIG. 1. Normal ovary of a 16-day rat embryo. Control to figs. 2, 3, 4, 5, 6, 7, 8, 9, 10. All the germ cells are of the oogonial type. Bouin-Allen and Feulgen. × 1200.

FIG. 2. Section through a 16-day embryonic rat ovary cultivated *in vitro* for 42 hr., showing the leptotene stage of meiosis. Note the fine strands of the chromatin. Bouin-Allen and Feulgen. × 1000.

FIG. 3. Section through the same ovary as that shown in fig. 2. The leptotene and synaptene stages of the meiotic prophase are seen. × 1000.

FIG. 4. Dividing oogonia in a 16-day embryonic rat ovary fixed after 42 hr. *in vitro*. Bouin-Allen and Feulgen. × 1000.

FIG. 5. Section through a 16-day embryonic rat ovary after 50 hr. cultivation showing nests and cords of cells which have developed *in vitro*. Note the healthy appearance of the ovary. Bouin-Allen and Feulgen. × 140.



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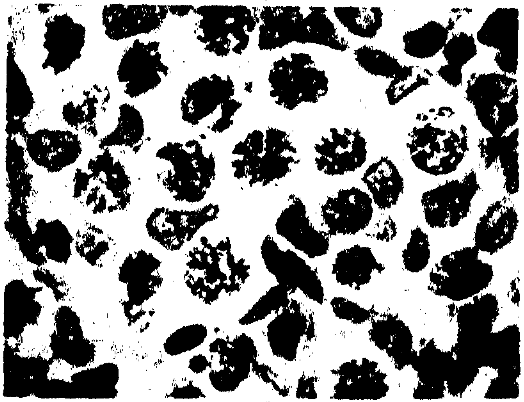

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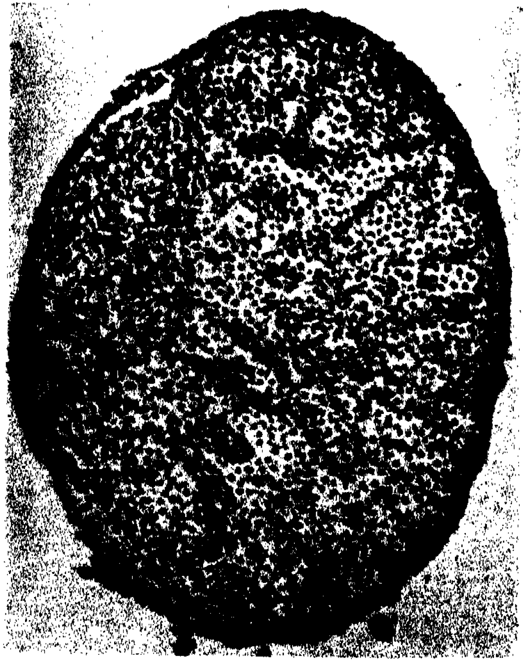

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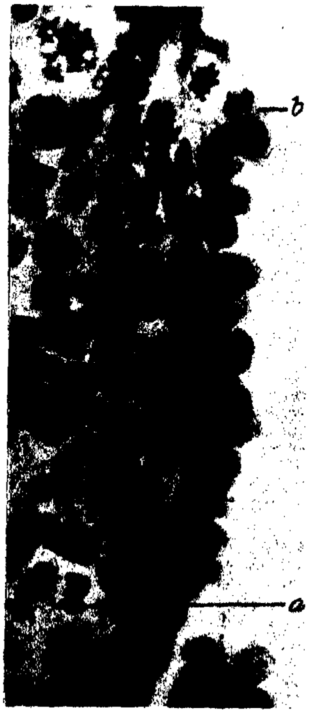

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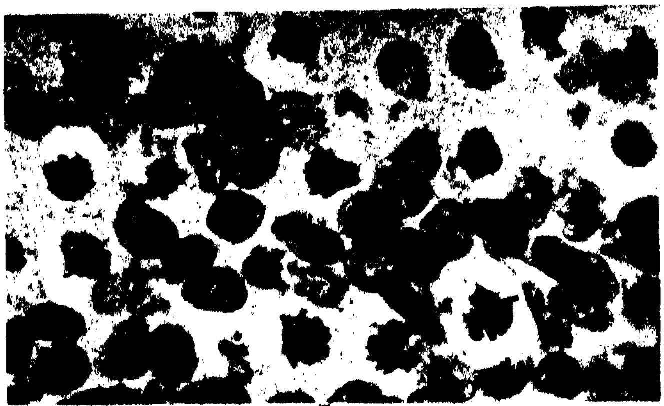

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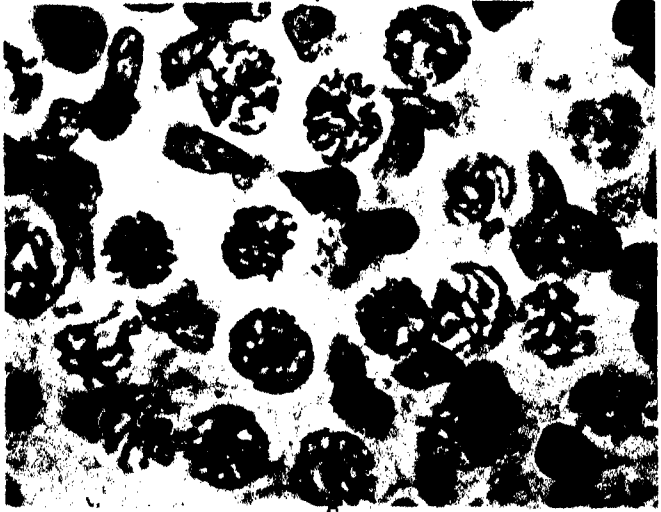

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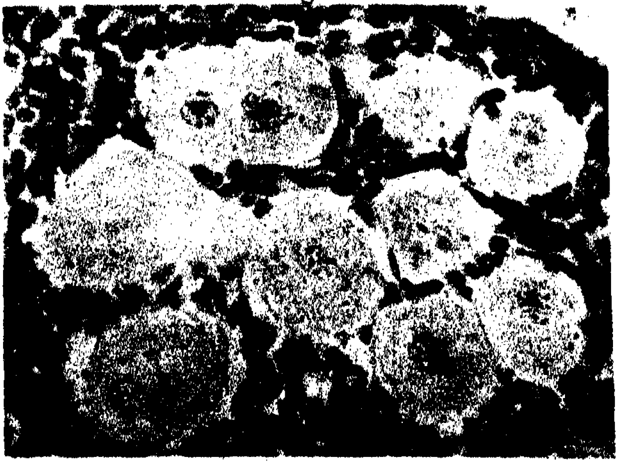

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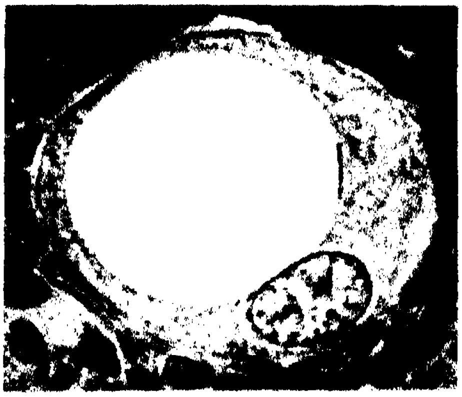

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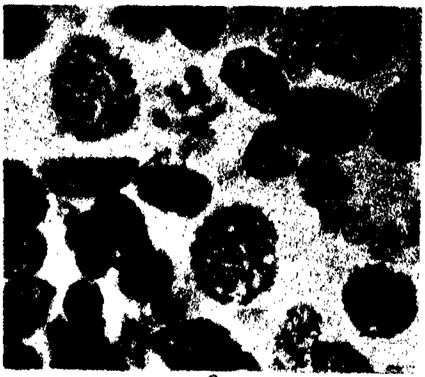

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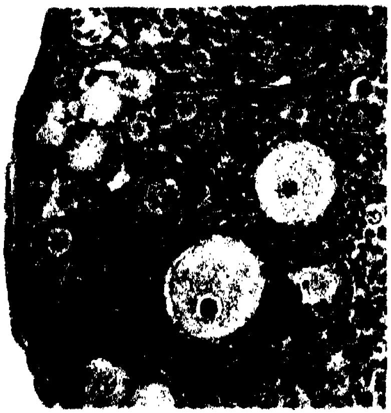

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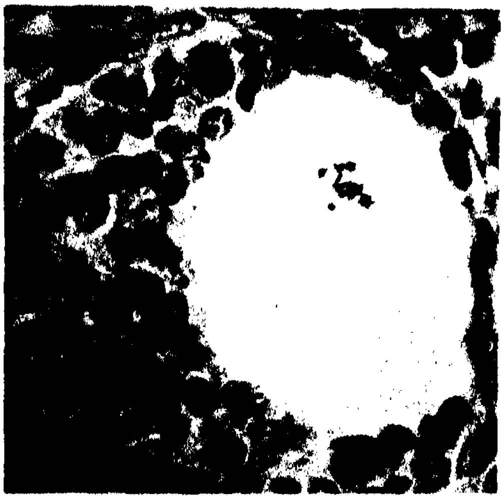

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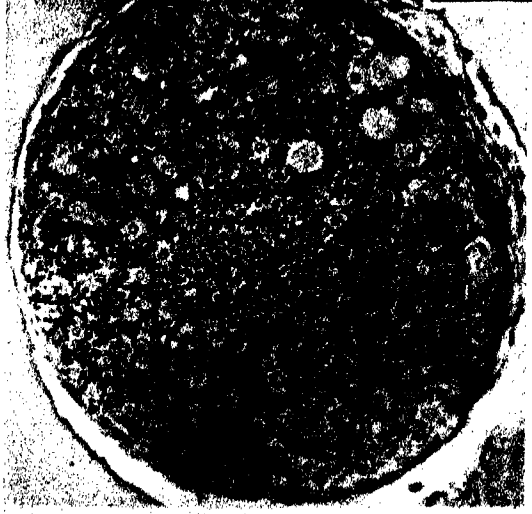

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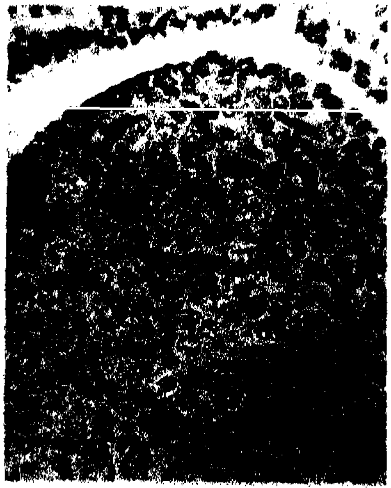

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Fig. 6. The abnormal division of two adjacent germ cells (*a*) is seen (lower clumped metaphase figure in a different plane of visibility), and the normal division of a somatic cell (*b*) in the germinal epithelium. From a 16-day embryonic rat ovary fixed after 50 hr. *in vitro*. Bouin-Allen and Feulgen. × 1000.

Plate 10

Fig. 7. Section through a 16-day embryonic rat ovary after 71 hr. cultivation showing the oocytes in the deep contraction stage (synizesis) of the meiotic prophase. Bouin-Allen and Feulgen. × 1000.

Fig. 8. Section through a 15–16-day embryonic rat ovary after 6·16 days *in vitro*. Numerous oocytes in the pachytene phase of the meiosis. Bouin-Allen and Feulgen. × 1200.

Fig. 11. Section through a 16-day mouse ovary after 19 days' cultivation. Nearly full grown ova are closely packed in the region of the original nests and cords. An incomplete single layer of cells surrounds some of the ova; the other ova are naked. Bouin-Allen and Meyer's haematoxylin. × 400.

Plate 11

Fig. 9. Section through a 15–16-day embryonic rat ovary after 7·2 days *in vitro*. Oocytes in the diplotene phase of the meiosis are shown. Bouin-Allen and Feulgen. × 1200.

Fig. 10. A large ring-like ovum in a 16-day embryonic rat ovary grown *in vitro* for 24 days. Bouin-Allen and Feulgen. × 1000.

Fig. 12. Section through the ovary of a 19–20-day embryonic rat after 21 days *in vitro*. Typical Graafian follicles have developed during cultivation and the nuclei of the oocytes have passed into the resting stage. Bouin-Allen and Meyer's haematoxylin. × 180. See fig. 13.

Fig. 13. Normal 19–20-day embryonic rat ovary. Control to fig. 12. × 290.

Fig. 14. Section through the same explant as that shown in fig. 12. Numerous residual ova are seen. × 100.

Fig. 15. Section through the ovary of a 4-day (post-embryonic) rat ovary after 9 days' cultivation *in vitro*. Bouin-Allen and Meyer's haematoxylin. × 640.

612.796 : 595.372

The receptive mechanism of the background response in chromatic behaviour of Crustacea

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(*Communicated by Lancelot Hogben, F.R.S.—Received* 21 *December* 1937)

[Plate 12]

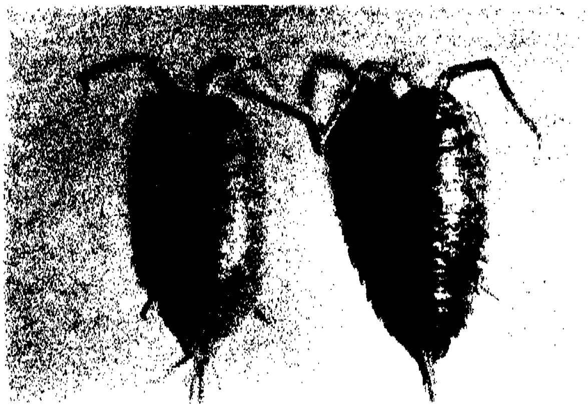
1. Introduction

Recent work (Koller 1925; Perkins 1928) on crustacean colour change has provided suggestive evidence of humoral co-ordination. Before it can be accepted as conclusive, it is necessary to examine more carefully the normal reactions, the relevant stimuli and the receptive mechanisms involved. The present communication is mainly concerned with one aspect of the latter.

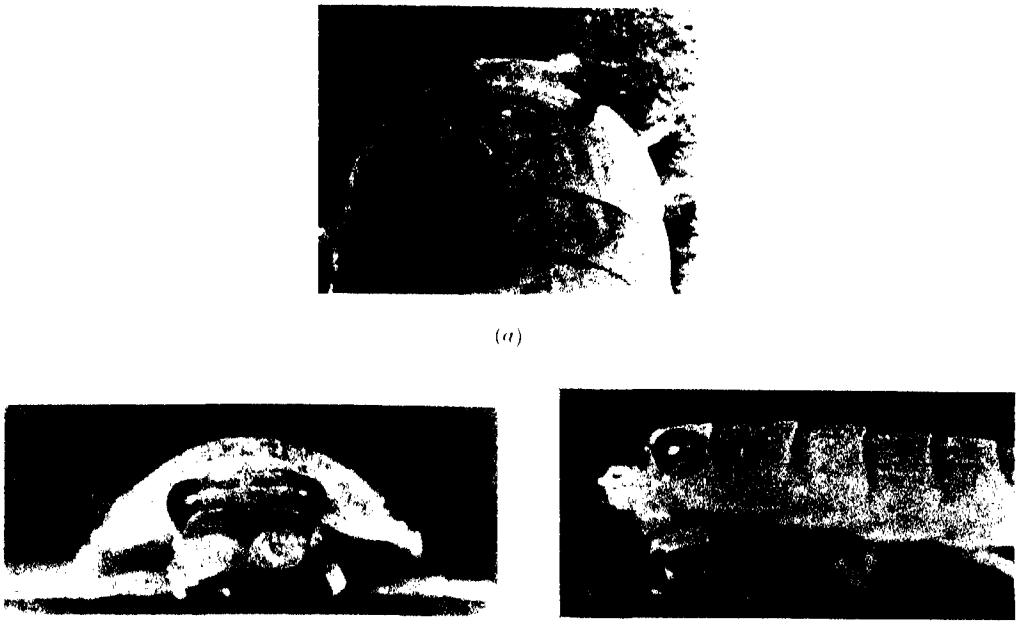
That *Ligia oceanica* responds by colour change to "background", i.e. light scattered from its immediate surroundings, and that the response depends on the eye has been recorded in a note by Tait (1910). This animal has been selected for further investigations for the following reasons:

(*a*) The eye is sessile. Hence the field of vision can be controlled more readily than is possible in decapod and in schizopod species on which most previous work dealing with crustacean colour change has been based.

(*b*) The pigmentary effector system of *Ligia* is relatively simple. In many crustacea, colour change is complicated by the presence of compound chromatophores with different types of pigment, all of which play an important part in the process. In *Ligia* separate xanthophores and melanophores very much like those of *Xenopus* are found in many regions. Elsewhere complex chromatophores of the type usually regarded as characteristic of Crustacea seem to be merely a super-position of melanophores on the xanthophores. The latter play a very insignificant part in colour change and, in fact, are only visible when the chromatophores are contracted. Plate 12 shows two individuals after illumination on white and black backgrounds and the eyes of *Ligia*.



Ligia oceanica (L.). Two individuals photographed after illumination on white and black backgrounds.



(*a*)

(*b*) (*c*)

Ligia oceanica (L.). Three views showing the eyes.

(*Facing p.* 251)

2. METHODS AND MATERIALS

For purposes of recording changes in contraction and expansion of the melanophores and equilibrium values the melanophore index (μ) introduced by Hogben and his collaborators was used. On this scale pin-point contraction is denoted by 1·0 and maximal expansion by 5·0. Readings were taken from the posterior region of the body. The animal was held between two microscope slides bound firmly together by an elastic band. For the blinding experiments the eyes were first covered with Whitehead's varnish and then painted over with the black enamel. The varnish provided a rough surface for the enamel which otherwise peeled off rather easily. Animals fresh from the shore were used for each experiment. They were kept in tin containers painted on the inside with white or dull black enamel. It was not possible to keep an absolutely constant temperature throughout the experiments but the variation was not great, the range being 15 ± 2° C. Every figure given in what follows is based on a mean of twenty-four animals.

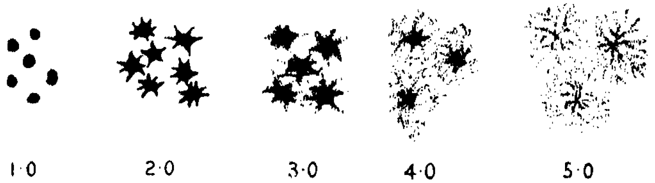


FIG. 1. The melanophore index (diagrammatic).

3. THE PRIMARY AND SECONDARY RESPONSER

In nature *Ligia* exhibits striking differences of coloration ranging from black to a light mottled grey. The experiments in Table I indicate that this range of colour is partly due to the amount of light reflected from its field of vision. On a black (light-absorbing) background illumination of the intact animal causes expansion of the melanophores. On a white (light-scattering) background the melanophores contract. This brings about pallor to an extent never seen in nature (Plate 12). This is only true if the eyes are exposed. Animals blinded by painting over the eyes with black enamel show a stage of melanophore expansion less than in seeing animals on a black background and considerably more than in non-blinded animals on a white background. In darkness the melanophores assume an intermediate condition less than that of blinded animals in light, the value of the melanophore index being 2·7. Two important facts thus emerge from this preliminary study of the response of *Ligia* to illumination:

(*a*) The white and black backgrounds initiate reverse responses. These responses are quite unlike either that of blinded animals, or that of normal animals in darkness.

(*b*) There is a direct or "primary" response of the melanophores independent and distinct from the background or "secondary" response for which the eye is the receptor organ. Blinded animals in dim light have a lower melanophore index than blinded animals in bright light. The dim light was produced by the insertion of a muslin screen. Non-blinded animals when kept on either white or black backgrounds also have a lower melanophore index when the overhead illumination is reduced. In *Ligia* the "primary" response is much greater than in *Xenopus* (Hogben and Slome 1931).

TABLE I. NORMAL CHROMATIC BEHAVIOUR

Animal	Background	Bright light	Dim light	Total darkness
Normal	Black	5·0 ± 0·00	4·6 ± 0·08	2·7 ± 0·10
Blinded	Black	4·2 ± 0·07	3·9 ± 0·07	2·7 ± 0·10
Normal	White	1·7 ± 0·08	1·4 ± 0·06	2·7 ± 0·10
Blinded	White	4·2 ± 0·06	3·9 ± 0·09	2·7 ± 0·10

4. TIME RELATIONS OF CHROMATIC FUNCTION

If the background to which the animals are adapted is suddenly reversed they gradually readapt themselves. Fig. 2 shows curves for colour change following reversal from a white to a black background and vice versa. The curves differ strikingly not only in direction but also in character. From a white to a black background the time curve shows a gradual rise and equilibrium is not reached until after six hours. From a black to a white background the change is very rapid compared with the reverse change and equilibrium is attained in an hour and half. It is difficult to believe that a mechanism with such a delayed time reaction is purely nervous. Further information about the co-ordinating mechanism can be gained from a comparison between: (*a*) the time relations (fig. 2) for background reversal and (*b*) the equilibrium intervals for transition from illumination on a white and black background to darkness and vice versa. The rates of colour change resulting from transferring *Ligia* from darkness to black or white background in light and from white or black background in light to total darkness are shown in fig. 3.

If the coordinating mechanism involves the production of one or more hormones, the significance of the time relations of chromatic response in

Ligia may be examined by elaborating an argument advanced elsewhere by Hogben and Slome (1936). A one-hormone hypothesis implies either (*a*) a melanophore-contracting hormone, *W*, whose production is inhibited

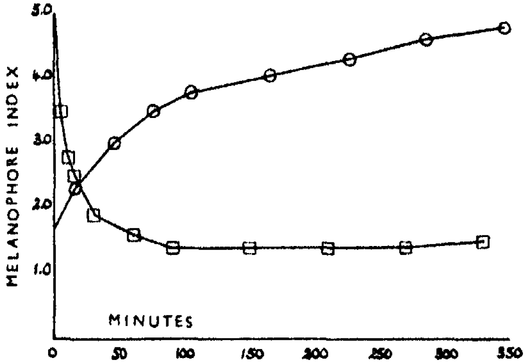


Fig. 2. Time curves of reversal from white to black and black to white background.

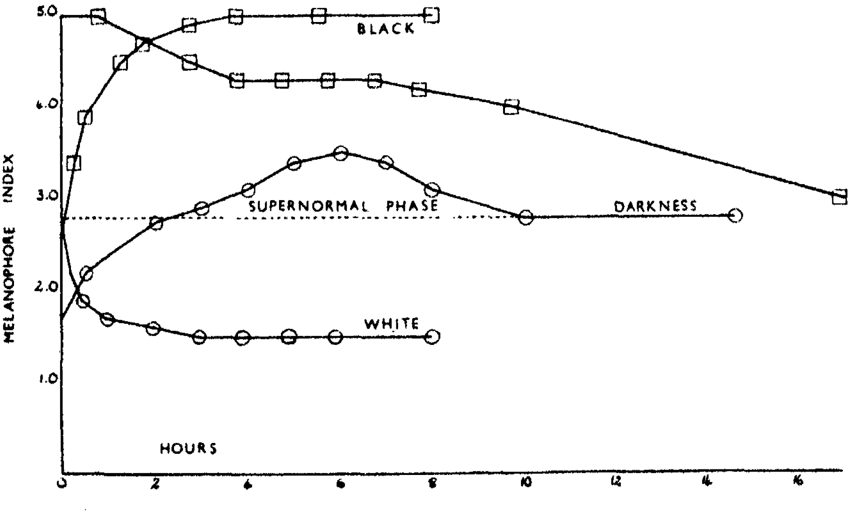


Fig. 3. Time curves of transition from black and white backgrounds to darkness and vice versa.

on a black background or (*b*) a melanophore-expanding hormone, *B*, whose production is inhibited on a white background. The truth of either postulate can be tested by the time reactions summarized in Table II where the symbols used are self explanatory. Thus ${}_bT_w$ means time taken for animals

TABLE II

Time of process in hours	Approximate melanophore range
${}_bT_w = 2$	4·5–1·5
${}_bT_d = 20$	4·5–3·0
${}_dT_w = 3$	3·0–1·5
${}_wT_b = 6$	1·5–4·5
${}_wT_d = 10$	1·5–3·0
${}_dT_b = 4$	3·0–4·5

already equilibrated to a black background to equilibrate on a white background, ${}_bT_d$ time taken for animals equilibrated in a black background to equilibrate in darkness, etc. If a melanophore-contracting hormone is the active agent ${}_wT_b$ is the time occupied in eliminating the contracting hormone by excretion, oxidation, hydrolysis or otherwise, *after the stimulus due to the white background has subsided*; and it involves eliminating a sufficient quantity to shift the melanophore index from about 1·5 to 4·5. Likewise ${}_wT_d$ involves eliminating only sufficient to shift it from about 1·5 to 3·0, and ${}_dT_b$ involves eliminating only sufficient to shift it from about 3·0 to 4·5. Hence ${}_wT_b$ must be greater than either ${}_wT_d$ or ${}_dT_b$. This is not so. Likewise, if a melanophore-expanding hormone is the active agent ${}_bT_w$ must be greater than either ${}_bT_d$ or ${}_dT_w$. This is not so. Hence a one-hormone hypothesis must be rejected.

A two-hormone hypothesis implies that two hormones with different rates both of production and elimination are simultaneously competing for effective relative excess. So it is open to neither of these objections and furthermore explains a striking feature of fig. 3. A two-hormone hypothesis implies that ${}_wT_d$ corresponds to the elimination of an *excess* of W and ${}_bT_d$ to the elimination of an *excess* of B. Since ${}_wT_d$ is much less than ${}_bT_d$, the elimination of B must be much slower than the elimination of W. Hence there should be a phase in which the quantity of B relative to W in the transition from white background to darkness is greater than it will eventually become. Thus there will be a *supernormal phase.*

5. DIRECTION OF INCIDENT ILLUMINATION

In some Crustacea (e.g. *Phronema*) there is a morphological differentiation of the compound eye into separate upper and lower groups of ocelli. The simplest explanation of the fact that seeing animals in equilibrium with a black background in light are darker than blinded animals, is that stimulation of different parts of the eye calls into action one or other of two hormones (B and W). This explanation of the same paradox has been proved true of Amphibia (Hogben and Slome 1936), and there is evidence that localized differentiation of the retina (Sumner 1923) is involved in the background responses of teleosts.

Keeble and Gamble (1904, 1905) were not able to demonstrate localization of different groups of ocelli involved in the background responses of the crustacean genera *Hippolyte* and *Macromysis*. From the geometry of the eye (Pl. 2) and the posture of *Ligia* we may distinguish broadly between: (*a*) the dorsal part of the eye (*D*) directly accessible to overhead illumination; and (*b*) a latero-ventral portion (*L* and *V*) accessible to light scattered from immediate surroundings. If the two-hormone hypothesis is correct we may suppose (fig. 4) that illumination of *D* is responsible for the liberation of *B* while illumination of *L* or *V* is responsible for the liberation of *W*. One way of testing this is to record the effect of reversing the normal direction of incident illumination, as Keeble and Gamble tried to do. The apparatus used by the writer is indicated in fig. 5. The containers were either covered by, or inverted over, a plate of opal glass under conditions of equal overhead and inferior illumination. The containers used were (*a*) wholly black, (*b*) with black sides and white bottom (i.e. top when inverted), (*c*) with white sides and black bottom (i.e. top inverted). The results of these experiments are recorded in Table III which gives the equilibrium value for the melanophore index.

6. DELIMITING THE RETINAL FIELD

The foregoing series of experiments clearly establish the division of the eye into a dorsal and a latero-ventral regions respectively responsible for the black and white background effects. When only the dorsal region is stimulated by overhead illumination the value of μ is maximal. When only the latero-ventral region is stimulated by inferior illumination the value of μ is minimal. The possibility of establishing more complete pallor encouraged the attempt to delimit the effective field of the two regions more precisely. If a white container with black bottom and white sides is inverted as in the experiment recorded in the bottom row of Table III, some of the dorsal region is inevitably illuminated by light scattered from the sides. Presumably the condition for maximum pallor is to ensure illumination of the entire latero-ventral group of ocelli without scattering any light on the dorsal group.

With this end in view the experiment last mentioned was modified by subjecting the animals to inferior illumination in inverted containers with a

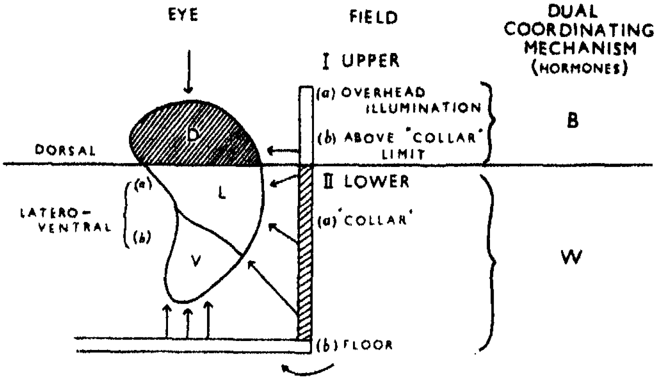


FIG. 4. Composite diagram showing the nomenclature used.

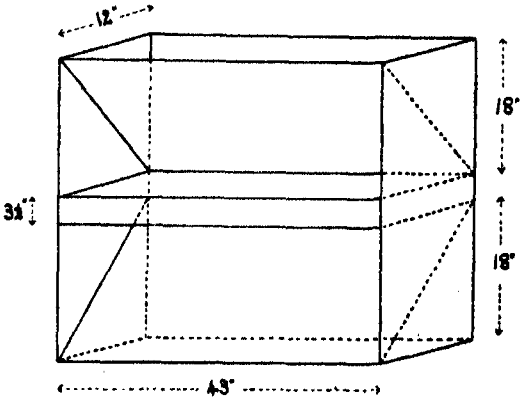


FIG. 5. Apparatus for providing equal illumination from above and below. The containers (3½ in.) high were placed between the two opal glass shelves and illuminated by means of reflected light from white paper. The lamp was placed 1 ft. away from the centre of the shelves.

TABLE III

Direction of illumination	Area stimulated	Container			μ
		Top	Sides	Bottom	
Above	D	White	Black	Black	4·7
	$D+L$	White	White	Black	2·5
	$D+V$	White	Black	White	2·4
Below	V	Black	Black	White	1·8
	$L+V$	Black	White	White	1·4

white collar of varying depth. This is the first arrangement shown in fig. 7. The equilibrium value for the melanophore index obtained with collars of different sizes is indicated in the accompanying graph (fig. 6).

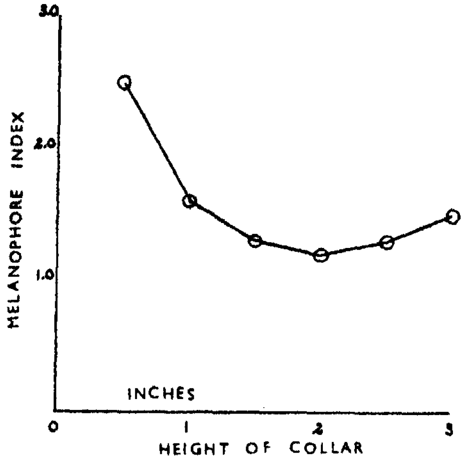


FIG. 6. Graph showing the values of μ for different heights of collar.

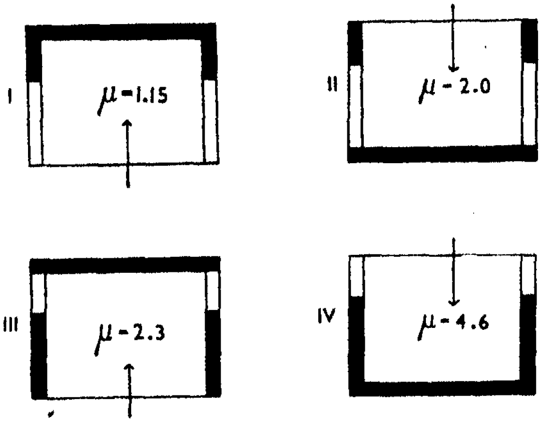


FIG. 7. Diagrams of containers used for delimiting the retinal field with appropriate values of μ. (I) and (III) have inferior illumination and (II) and (IV) superior illumination. The black and white regions indicate how the containers have been painted.

On the basis of this experiment a height of 2 in. in a cylindrical vessel of height 3½ in. and diameter 9 in. was taken as the limit of effective field from which light scattered upwards can effect the D group of ocelli. In a new series of experiments the effective field of the D region was delimited by the roof and a collar of 1½ in., while the effective field of the latero-ventral region was delimited by the floor and a collar 2 in. high. Four arrangements, two for overhead and two for inferior illumination, are shown in fig. 7 with the observed values for the melanophore indices.

The results of these experiments are summarized in fig. 8 which exhibits the values of μ obtained when the greatest possible care is taken to restrict illumination to the groups of ocelli specified as D, L, V. In this figure the black area is the area stimulated by direct or scattered rays.

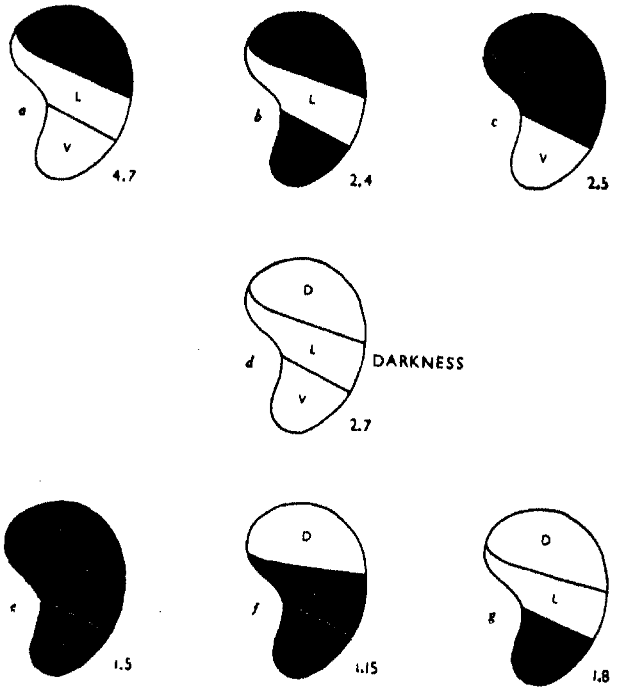


FIG. 8. Chart of combined experiments showing the values of μ when illumination is restricted to different groups of ocelli D, L and V. The regions illuminated are painted black.

7. SELECTIVE ELIMINATION OF OCELLI

Owing to the anatomical peculiarities of the compound eye, it is possible to apply a direct test to the conclusions established in the last two sections. By blinding only the dorsal part (D) of the eye the animals should give the same response as totally blinded on a black background and a lower melanophore index on a white background than either blinded animals or seeing animals. With only the latero-ventral part ($L + V$) of the eye blinded the response on both black and white backgrounds should be the same as for seeing animals on a black background. Table IV exhibits the results of these experiments. The melanophores of the two classes are compared with those of seeing animals (*O*) and of totally blinded animals ($D+L+V$). Despite the fact that such experiments must be somewhat crude, the results obtained provide conclusive proof for the view advanced on the basis of evidence set forth in the two foregoing sections. Blinding experiments of that kind are easy to perform. The visible results are so striking that they have been included as a departmental demonstration in the course for first-year students.

TABLE IV

Group of ocelli eliminated	Background: Black	Background: White
0	5·0	1·7
D	4·0	1·5
$L+V$	4·6	4·5
$D+L+V$	4·2	4·2

8. MONOCHROMATIC ILLUMINATION

In their analysis of the dual receptor mechanism of Amphibian colour change Hogben and Slome were not able to appeal to the direct method used in the foregoing section to confirm the conclusions established by varying the direction of incident illumination. They were, however, able to obtain independent evidence for the existence of two different receptive elements from another source. By illuminating *Xenopus* on black and white backgrounds with rays of different wave-lengths they were able to show that the photo-receptors respectively for the black and white background responses are sensitive to different regions of the spectrum. The following experiment was undertaken to see if a similar sensitivity exists in the eye of *Ligia*.

Table V shows the different Wratten filters used with the appropriate wave-length ranges, flux transmission, and relative flux. The relative power for equal flux has been calculated to indicate lighting conditions. Thus if a 100 W lamp is used for filter No. 62, a 9 W lamp must be used for filter No. 70. To compensate for the difference in the relative power for equal flux two 25 W lamps and two 200 W lamps each pair being of equal intensity were used. With the exception of the experiment in which filter No. 29 was used the range of equal flux lies approximately within the intensity range of the lamps. Simultaneous experiments with different filters were carried out in two light-proof boxes. In order to obtain complete radiation inside the boxes it was necessary to have the light not more than three inches from the filter and therefore a water bath had to be placed between lamp and filter.

If the recorded results in Table VI were due entirely to the visual response they would suggest that both *D* and *L* + *V* groups of ocelli are more sensitive to the blue end and that there is a mid-region (green-yellow) to which the *L* + *V* group is less receptive than to either the blue or red end of the spectrum. It would be rash to draw this conclusion without regard to the primary response, and data obtained with blinded animals did not conclusively demonstrate how far the high melanophore indices in the second and fourth columns are due to differential sensitivity of the melanophores to direct illumination.

TABLE V

No. of filter	Range	Flux transmission	Relative flux	Relative power for equal flux
49 (blue)	400–490	3,981	149	67
62 (green)	520–570	2,675	100	100
73 (yellow)	560–620	3,056	122	82
29 (red)	610–700	85,350	3,200	3
70 (red)	650–700	28,652	1,146	9
89 (red)	650–700	23,500	940	11

TABLE VI. MONOCHROMATIC LIGHT

	200 W lamp		25 W lamp	
No. of filter	Black	White	Black	White
49	4·7	1·4	4·4	1·2
62	4·7	2·0	4·5	1·8
73	4·7	2·1	4·5	2·0
29	4·6	2·2	4·4	1·8
70	4·5	1·9	4·3	1·6
89	4·5	1·9	4·2	1·5

9. THE SIGNIFICANCE OF THE SUPERNORMAL PHASE

Evidence for a double humoral co-ordination of chromatic behaviour derived from the supernormal phase in *Ligia*, as set forth in § 4, may now be explored more fully from a new angle. If the supernormal phase depends on the relative amounts of *B* and *W*, any alteration of the proportion of one to the other should bring about corresponding differences in the supernormal phase. To test this, animals that had been kept on a black background for two days were divided into two sets. One set was placed in a white container so that the whole of the eye ($D+L+V$) received illumination for 16 hr. The other set was illuminated for the same time in the way described in § 6. That is to say, only the D and V parts of the eye were illuminated. Both sets were then transferred to the dark room, where the melanophore index was recorded at regular intervals.

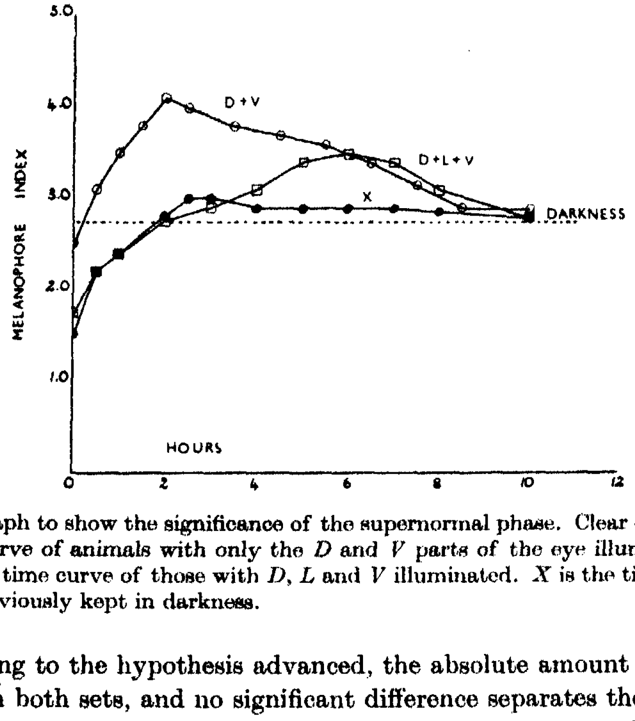


FIG. 9. Graph to show the significance of the supernormal phase. Clear circles mark the time curve of animals with only the D and V parts of the eye illuminated and squares the time curve of those with D, L and V illuminated. X is the time curve of animals previously kept in darkness.

According to the hypothesis advanced, the absolute amount of B must be large in both sets, and no significant difference separates them on this account. This should not be true of W. The absolute amount of W should be greater when the area illuminated is $D+L+V$ and less when the area illuminated is $D+V$. Such a difference is reflected in the magnitude and duration of the supernormal phase; as shown in fig. 9.

This difference would be expected if the two-hormone hypothesis is correct. Experiment has already shown that W must be eliminated more rapidly than B. If there is relatively less W, the latter will be exhausted before B has greatly diminished. The effect of this will be to make the ratio $B:W$ greater during the process of elimination. Hence the supernormal phase will have a higher peak. Fig. 9 shows that the supernormal phase for the $D+V$ group is much more pronounced and reaches its peak more quickly than the supernormal phase of the $D+L+V$ group.

By lowering the amount of B to a minimum, it should also be possible to reduce the supernormal phase. According to the two-hormone hypothesis the effect of transferring an animal from darkness to a white background is to produce a rapid discharge of W in excess of B which accumulates very slowly and does not reach the threshold for $\mu > 2$. Animals, previously kept on a white background for a short period such as 16 hr., will have accumulated more B if they have been previously exposed to a black background than they would have if they had been previously kept in darkness. To test this, the behaviour of the first set of the pair described in the preceding paragraphs was compared with a third set (X) kept for 2 days in darkness before exposure in a white container for 16 hr. as before. If the two-hormone hypothesis is correct they should have less B, and therefore a great reduction of the supernormal phase. Fig. 9 shows that this is so.

This investigation was undertaken at the suggestion of Professor Lancelot Hogben to whose help the author is indebted. Acknowledgment is also due to the Carnegie Research Fund for Scottish Universities.

10. SUMMARY

1. The pigmentary effector system of *Ligia* consists of melanophores and xanthophores. They are separate in some regions and in other regions superimposed so as to form complex chromatophores.

2. The melanophores are the chief agents of visible colour change. Their response to illumination is partly direct and partly controlled by the eyes.

3. The primary response is of the type described for decapods and mysids by Keeble and Gamble and for vertebrates by Laurens (1915) and by Hogben. Intense illumination increases the diffusion of pigment.

4. The secondary or visual response to background is also of the type described for *Macromysis* and vertebrates. Overhead illumination in a black container makes the animal dark, and overhead illumination of an animal in a white container makes it pale. Blinded animals are not so dark as non-blinded animals kept on a black "background". In darkness they are still more pale owing to elimination of the primary response.

5. The time relations of the background response show that it is controlled by two hormones. The existence of a "supernormal phase" in the

transition from white background equilibrium reinforces this conclusion. Experiments which show how the supernormal phase may be accentuated or eliminated also support this conclusion.

6. The effects of (*a*) varying the direction of incident illumination (*b*) limiting the light scattered on to the eye, make it possible to distinguish two groups of ocelli. One (dorsal) is accessible to direct overhead illumination and is responsible for initiating the discharge of the hormone which evokes melanophore expansion. The other (latero-ventral) picks up light scattered from the immediate surroundings and is responsible for initiating the discharge of the hormone which evokes melanophore contraction.

7. The effects of painting the whole eye, the ocelli of the upper half only, or the ocelli of the lower half alone, directly confirm this conclusion.

8. The blue end of the spectrum is most effective for evoking the visual or secondary response.

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Chromatic behaviour of elasmobranchs

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[Plate 13]

1. Introduction

The first recorded observations on the pigmentary effector system of elasmobranchs were negative. Schaefer (1921) recorded that *Raia clavata* and *R. batis* maintained a dark coloration on both white and black "backgrounds", i.e. immediate visual field. Parker (1933) repeated these tests on *R. erinacea* and described visible paling on a white background and darkening on a black background. In collaboration with Porter (1934) the same author demonstrated a similar reaction in the dogfish, *Mustelus canis*. The observations on colour changes in response to background were extended to elasmobranch fishes of English waters in 1936 when Waring described similar responses in *Scyllium canicula* and Hogben in *Raia brachyura*, *R. maculata*, *Rhina squatina* and *Scyllium* sp. In all these species it has been explicitly recorded that the natural response is a slow one.

With regard to the receptive mechanism involved in these changes, it is generally agreed that discrimination between white and black backgrounds is visual. The work of Lundstrom and Bard (1932), Hogben (1936*a*) and Waring (1936) on excision of the separate lobes of the pituitary and the injection of extracts, clearly established that the dark phase in all elasmobranchs is due to the activity of the neuro-intermediate lobe of the pituitary. Transfusion of blood from animals kept on different backgrounds has provided independent evidence for the influence of a blood circulated hormone (Parker and Porter 1934; Waring 1936).

Concerning the co-ordinating mechanism which controls the pale phase (contracted melanophores) there is less uniformity of opinion. From a study of the speed of natural change and experiments on excision of the anterior lobe of the pituitary of a number of English species, Hogben (1936*a*) and Waring (1936) concluded that there is a positive humoral control of paling localized directly or indirectly in the anterior lobe of the pituitary. No valid criticisms have been made of these experiments or of their interpretation. On the other hand experiments on the section and faradic stimulation of nerves in the American species *Mustelus canis* and *Squalus acanthias* led Parker and Porter (1934) and Parker (1935, 1936*a*, *b*, 1937) to conclude that control of melanophore contraction is largely due to direct innervation of the melanophores. The possibility of direct nervous control of melanophore contraction in elasmobranchs is not confirmed by other work on severance and stimulation of nerves carried out by Young (1933), Waring (1936) and Wykes (1936). There are serious objections to the conclusion which Parker and his co-workers have drawn from their observations. The chief one is that a slow reaction is not consistent with what is known of other reactions which have a nervous basis. Another is that the experiments performed by Parker and his co-workers do not exclude the intervention of vaso-motor disturbances which would bring about local humoral variations (Hogben 1936*b*).

Despite these objections Parker has continued to ignore the possibility of a positive humoral control of paling in elasmobranchs. Curiously enough he concedes the possibility of a decreased secretion by the neuro-intermediate lobe during the transference from black to white background (Parker 1937). For this there is no direct evidence. Parker (1937) now claims that excision of the anterior lobe of *Mustelus* has no effect on the natural colour response. In view of his inability to obtain pronounced paling on removal of the whole pituitary of *Squalus acanthias*—an animal which in fact undergoes a most striking change (fig. 8)—his observations on removal of the anterior lobe alone must be viewed with reserve.

Hitherto those who have claimed a predominantly direct nervous control of melanophores (as in teleostei and reptiles) or a predominantly reflex humoral control (as in amphibia, cyclostomes and crustacea) have never asserted that one and the same mechanism of co-ordination is universal. In Parker's writings a spurious universality has now been introduced by the term *neurohumoral* co-ordination. So used the term conceals an important distinction. Apart from evidence for direct nervous control or the secretion of specific hormones one difference between two classes of chromatic behaviour is beyond dispute. These two classes are distinguished by the time relations of colour change, which may complete itself within a few seconds or minutes (some teleostei and reptiles) or may occupy several hours or even days (amphibia and elasmobranchs). Recent work suggesting that the reaction at the nerve-muscle junction involves liberation of a drug-like substance, which Parker has incorporated in the generalized theory of "neurohumours", has no bearing on this broad distinction. That reactions controlled by nerves are generally quick is a fact beyond dispute. Whether nerves excite effector organs by the polarizing action of ions at the separating membranes or, as the new hypothesis postulates, by explosive liberation of organic excitants such as acetyl choline, does not affect the issue.

The present investigation had two objects:

(*a*) To extend the observations of Hogben (1936*a*) and Waring (1936) on the role of the anterior lobe of *Scyllium* in the natural pallor reaction.

(*b*) To test the role of the anterior lobe in the chromatic behaviour of one of the two American species for which direct nervous control of melanophore contraction has been so strongly advocated. For this purpose *Squalus acanthias* [= *Acanthias vulgaris*] was selected. *Mustelus canis* was unobtainable.

The experiments were carried out at the Biological Station at Port Erin while the author held the Herdman Scholarship of the Zoology Department at Liverpool University.

2. OPERATIVE TECHNIQUE

The elasmobranch pituitary has three easily separable lobes (fig. 1). The ventral lobe arises from the neuro-intermediate lobe at its junction with the pars anterior. In *Raia* it is free. In *Scyllium* and *Squalus* it comes into intimate contact with the floor of the cranium and is surrounded by the cartilaginous membrane of the floor. In *Raia* (Hogben 1936*a*) it is impossible to remove the neuro-intermediate lobe without the ventral. It is possible to leave the ventral lobe of either dogfish *in situ* with its blood supply intact. Even if the forward branches of the divided carotid are accidentally severed, blood will still pass through the ventral lobe into the interorbital sinus. Owing to the erratic behaviour ensuing after ventral lobe extirpation (p. 271) it is desirable (though not essential) to leave it behind when comparing the tolerance to pituitary extract injections (*a*) of animals with both anterior and intermediate lobes removed, and (*b*) of those with only the intermediate lobe removed.

Separate removal of the anterior and intermediate lobes is a simple procedure in all three animals. Comparison of dissections of several specimens with the account given by O'Donoghue and Abbott (1927) shows that either the anterior or the intermediate lobe can be removed without interfering with the blood supply to the other. I have confirmed this experimentally by severing the anterior from the intermediate lobe. This procedure does not interfere with normal chromatic behaviour. The operative procedure for *Raia* has been described by Hogben (1936*a*). The necessary cuts in the cartilage, following incision of the mucous membrane are indicated for *Scyllium* and *Squalus* in fig. 1. If the only objective is removal of the anterior lobe, the cuts need not be continued so far back. When the ventral lobe is to be removed the cuts in the cartilage must be extended further back. Some bleeding is then inevitable owing to the severance of the carotids and the interorbital sinus. In *Scyllium* it is convenient to make a superficial cut along the most anterior line indicated and then to hinge back a window of cartilage. Owing to the extreme thickness of the cartilage in *Squalus* it is easier to cut it right out. If the edges of the cut are bevelled, the piece will slip back easily into place and post-mortems indicate that it retains its position. The mucous membrane can be stitched with gut and will heal perfectly. A *Scyllium* on which I operated 9 months ago is still alive.

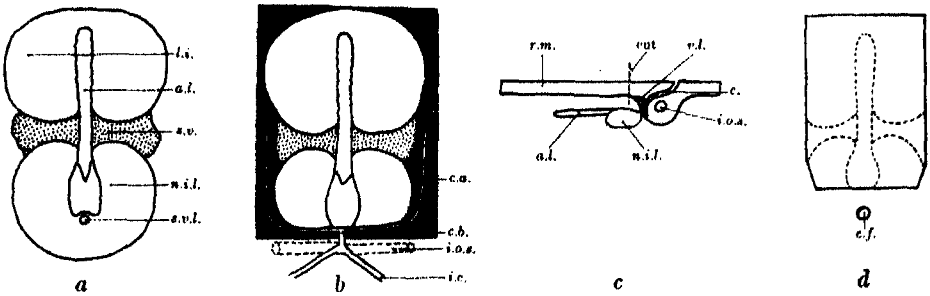

FIG. 1. Diagrams illustrating the operative technique. (*a*) Relation of the pituitary lobes. Ventral view. (*b*) The chief blood vessels to be avoided in the operation. (*c*) Side view showing the relation of the pituitary lobes to roof of the mouth. (*d*) Cuts to be made in the cartilage. Pituitary outline dotted in.

l.i.	lobi inferiores.
a.l.	anterior lobe.
s.v.	saccus vasculosus.
n.i.l.	neuro-intermediate lobe.
s.v.l.	stalk of ventral lobe.
c.	carotid.
c.a.	forward extension of carotid in cranial cavity.
c.b.	bifurcation of carotid in substance of ventral lobe.
c.f.	carotid foramen.
i.o.s.	interorbital sinus.
i.c.	internal carotid joining its fellow and entering the skull.
r.m.	roof of mouth.
v.l.	ventral lobe.

3. THE HISTOLOGICAL BASIS OF COLOUR

The melanophores of *Scyllium* have been previously described (Waring 1936). When brought into the laboratory *Squalus* is a uniform dark slate colour with a few white spots on its dorso-lateral surface. Usually it has a white fringe round the edge of the fins. Microscopic examination of its skin shows two types of melanophores which closely resemble those found in *Scyllium* (Waring 1936). As in *Scyllium* the dark epidermal melanophores are irregularly branched and usually associated with the bases of the scales. The dermal melanophores differ from those of *Scyllium* in two respects. First, their ramifications are coarser. So the exact outline of the expanded cell is easier to follow. Secondly, they vary in the actual pigment they contain. The majority have a brown pigment similar to that found in *Scyllium*. In others the pigment is distinctly orange-red. The behaviour of these two types of pigmentary effectors appears to be the same. I was unable to detect the presence of yellow cells similar to those of *Scyllium*. The presence of orange pigment in cells which are morphologically melanophores shows that there is no sharp line of demarcation between melanophores, xanthophores and erythrophores in Elasmobranchs. This also is suggested by the similar behaviour of xanthophores and melanophores where they are morphologically distinct (Hogben 1936*a*). It is also emphasized by the fact that *Squalus* has no xanthophores similar in form to those of *Scyllium* (Waring 1936). The white fringe to the fins of *Squalus* is not constant in width. It is caused by the absence of melanophores from that region. The last two rows of melanophores are frequently fully contracted, irrespective of the condition of those in the rest of the skin.

4. NATURAL COLOUR CHANGE

(*a*) *Scyllium canicula* (L.)

Scyllium pales in a white tank and darkens in a black one. These changes are brought about by the contraction and expansion of dermal and epidermal melanophores. The transition from one phase to another is a slow process (fig. 2). In a tank almost devoid of light an intermediate condition of the melanophores is attained. Transition from the intermediate phase of darkness to either the fully expanded state (illuminated black tank) or contracted state (white tank), takes at least as long as the transition from one extreme to the other (figs. 2, 3).

The speed of the change indicates a humoral basis. That the transition from pallor to the dark condition is due to a hormone (*B*) is undisputed (p. 264). Although no systematic experiments have been carried out to ascertain localization of separate receptive units in the Elasmobranch eye, it is clear that illumination of the basal elements alone brings about expansion and that illumination of the whole eye contraction. It is further known that in pale fish there is less *active B* in the blood than in dark fish (Waring, unpublished). This might be brought about (i) by withdrawal of *B*, or (ii) by the production of an antagonistic hormone (*W*) elsewhere. If (i) is correct transition from darkness to a black tank involves the secretion of less *B* than does transition from a white to a black tank, and should therefore take less time. This requirement can be conveniently represented as ${}_wT_b > {}_dT_b$. Similarly transition from darkness to a white tank involves the excretion or destruction of less *B*, therefore the expectation would be ${}_bT_w > {}_dT_w$. If (ii) is correct an animal equilibrated in a white tank will have *B* and *W* secreting fully, *W* overriding *B*. In a black tank *W* is reduced considerably and *B* rapidly gains ascendance. When the fish is replaced in a white tank, *B* remains constant and *W* rapidly increases.

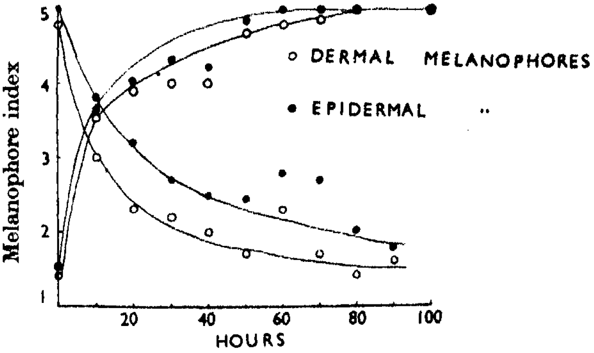

FIG. 2. *Scyllium canicula*. Reversal from white to black and black to white backgrounds.

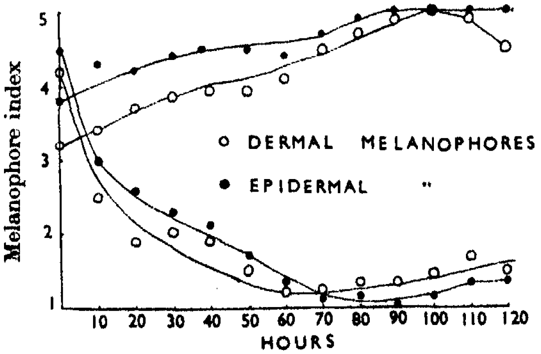

FIG. 3. *Scyllium canicula*. Transition from the condition in semi-darkness to that in illuminated white and black tanks.

During the transition from darkness to an illuminated white tank both B and W will be increased, W overriding B. Since two opposing reactions compete simultaneously, equilibrium might well be a lengthy process and it would not be surprising to find ${}_dT_b > {}_wT_b$ or that ${}_dT_w > {}_bT_w$. The curves do in fact show that ${}_dT_b$ and ${}_dT_w$ are at least equal to and probably greater than ${}_wT_b$ and ${}_bT_w$.

The preceding argument has been advanced by Hogben and Slome in studies on *Xenopus*. It has been applied in greater detail to the analysis of crustacean colour change by Smith (1938).

(*b*) *Squalus acanthias* L.*

In an illuminated black tank *Acanthias* maintains a dark slate colour, with both the dermal and the epidermal melanophores fully expanded. When placed in an illuminated white tank fish with intact eyes gradually pale. Microscopic examination then shows the contracted phase in melanophores of both types. Unlike those of *Scyllium* the eyes of *Squalus* are not provided with a resistant moveable eyelid. Consequently they are very liable to injury. Furthermore, slight injuries to the outer cornea, while being quite sufficient to destroy the natural responses, can only be detected on close inspection. Out of the total of eleven fish only four were obtained with perfect eyes. In a white tank two of these attained a fully contracted condition of most of the melanophores in terms of the melanophore index (Hogben and Gordon 1930). One attained 3 and one 3·5. With the exception of the last the macroscopic effect in all these fishes was very striking. Even with the last it was quite obvious. This white background could, however, only be produced if the tanks were heavily shaded from strong sunlight. I have sometimes found this inability to adapt in strong light in *Scyllium*. In the latter, however, there is considerable individual variation. Although systematic experiments with blinded animals have not been attempted, it is highly probable that this effect can be attributed to a very strong primary (i.e. direct or non-visual) response of the melanophores and that in bright light this is sufficient to overcome the secondary visual response to background (cf. Hogben 1936*a*, p. 144). Blind fishes were fully dark in either black or white tanks, a fact which suggests that there was sufficient light in either case to cause the maximum primary response. Parker (1936*a*) did not obtain a good natural pallor. This may have been due to the fact that he did not recognize the significance of a primary response or to visual defects of his specimens.

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* Also known as *Acanthias vulgaris* Risso.

Squalus takes at least 48 hours to become fully white-adapted, a time which is within the range reported for other elasmobranchs (Hogben 1936*a* for English species, Parker 1937 for *Mustelus*). It was not possible to obtain a comparable figure for full black-adaptation. When handled, pale fish rapidly became mottled and within ¼ hr. were fully dark all over. If such fish were returned to a white tank they slowly resumed their pallor. When placed in a dark tank they remained fully dark. Parker (1936*a*), in his paper on *Squalus*, makes no mention of this effect. All my own observations were made in the range of 9–12° C. and no attempt was made to vary the temperature.

5. THE SEPARATE REMOVAL OF THE VARIOUS LOBES OF THE PITUITARY AND THE INJECTION OF POSTERIOR LOBE EXTRACT

(*a*) *Scyllium canicula* (L.)

Removal of the neuro-intermediate lobe alone or together with the anterior lobe results in maximal contraction of the melanophores irrespective of the background. Removal of the anterior lobe alone results in maximal expansion of the melanophores irrespective of the background (Hogben 1936*a*; Waring 1936). Removal of the ventral lobe alone results in erratic behaviour (fig. 4). These findings from the separate removal of the various lobes of the pituitary together with the results from investigations on the injection of extract and blood transference show conclusively that the expanded phase of the melanophores is brought about by the activity of the neuro-intermediate lobe of the pituitary through the medium

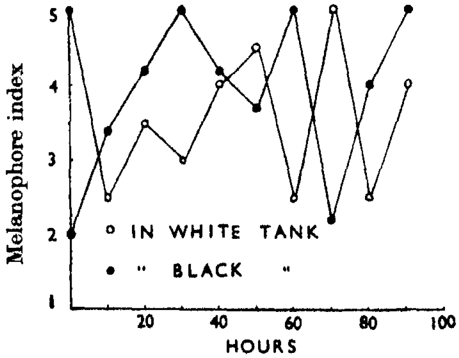

FIG. 4. *Scyllium canicula*. The erratic behaviour of two fish following the removal of the ventral lobe alone. Both had previously reacted normally.

of a blood circulated hormone. It is clear also that the anterior lobe is necessary for pallor—but injection of extract of the anterior lobe and implantation of the whole lobe into a dark fish have failed to show the presence of a hormone (*W*) antagonizing the *B* and thus causing pallor.

Hogben and Slome (1931, 1936) were able to demonstrate that a secretion of the *pars tuberalis* continuous with the anterior lobe of *Xenopus* antagonizes *B* in the blood by injecting *B* hormone into groups of normal, partially hypophysectomized and fully hypophysectomized animals. The relative sensitivity of the three groups showed quite clearly that the presence of the anterior lobe (or more strictly, the associated *pars tuberalis*) is antagonistic to injected *B*. They used groups of animals of equal weight and kept under constant conditions. Such a procedure with *Scyllium* was not possible owing to lack of tank space. Experiments of three types were performed by a modification of their method.

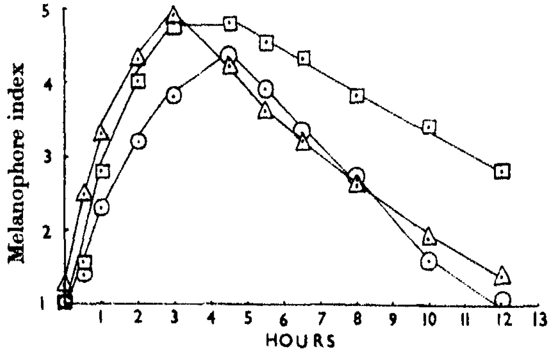

FIG. 5. *Scyllium canicula.* The response of normal and operated animals of equal weight to equal injections of *B* hormone. Each curve represents the average of three animals. White tank.

◬ Normal.
⊙ Intermediate lobe removed.
⊡ Anterior and intermediate lobes removed.

(i) Three animals of equal weight were selected and kept in the same tank. From one the neuro-intermediate lobe was removed, from another the intermediate and anterior lobes were removed, in the third the gland was left intact. Each was injected with the same quantity of *B* hormone, i.e. *pitressin* freed from pressor activity with normal caustic soda as prescribed by Hogben and Gordon (1930). Two other sets of three animals were treated in the same way. The mean figures are indicated in the accompanying graph (fig. 5).

(ii) Two animals which were not of the same weight were selected. From one the neuro-intermediate lobe was removed, from the other the intermediate and anterior lobes were removed. Each was injected with the same quantity of *B* hormone. The injections were repeated on successive

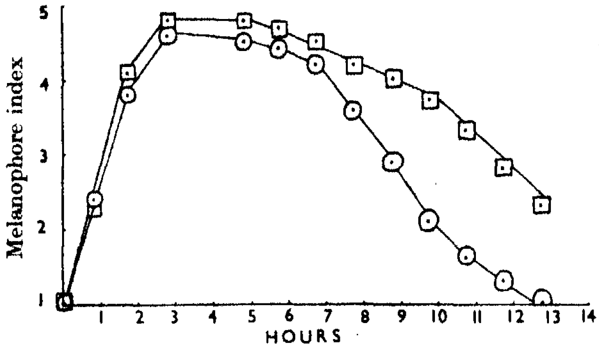

FIG. 6 (*a*). *Scyllium canicula*. The responses of two classes of operated animals to the injection of equal doses of *B* hormone. Each curve represents the average of several injections with five sets of animals. The animals were not all of the same weight. White tank.
⊙ Neuro-intermediate lobe removed.
⊡ Neuro-intermediate and anterior lobes removed.

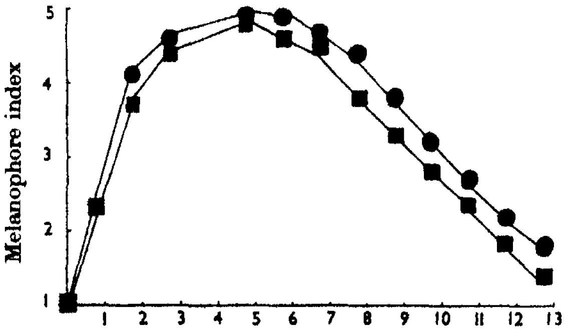

FIG. 6 (*b*). The responses of the same animals as fig. 6 (*a*), following the removal of the anterior lobe from group ⊙ now written ● and a dummy operation on group ☐ now written ■. White tank.

days. The anterior lobe was then removed from the animal which had previously lost its intermediate lobe and the incisions were reopened and closed in the other from which both lobes had previously been removed. The injections were repeated on successive days. This procedure was carried out on five sets of two animals. The results are plotted in figs. 6 (*a*) and 6 (*b*).

(iii) Three animals were kept in the same tank and injected with *B* to estimate the individual variations. The intermediate lobe was then removed from one, the anterior and intermediate from another, the gland being left intact in the third. The three were then injected with a *B*-containing extract

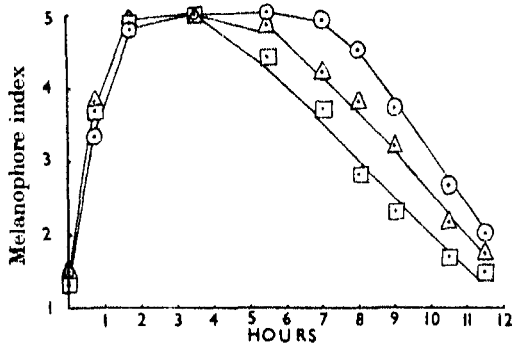

Fig. 7 (*a*). The responses of two sets of three normal animals to injections of equal doses of *B* hormone. The fish were not all of the same weight. White tank.

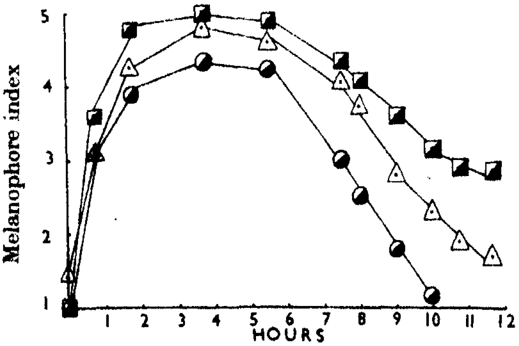

Fig. 7 (*b*). The responses of the same animals as fig. 7 (*a*), following the removal of the anterior lobe from set ⊙ (now written ◐) and the anterior and neuro-intermediate lobe from set □ (now written ◪). Each curve represents the average of several injections. White tank.

on successive days. Finally the anterior lobe was removed from the animal which had previously lost its intermediate lobe and the incisions of the other operated animal were reopened and closed. Further successive injections were then given, and the same procedure was carried out with a second batch of three animals. The results are shown in fig. 7 (*a*), (*b*), (*c*).

Fig. 7 (c) exhibits a feature which is remarkably constant. The animals from which both anterior and intermediate lobes had been removed at the first operation showed greatly increased tolerance to *B* after the second operation. In spite of this, the animals from which the neuro-intermediate lobe was removed showed *less* tolerance to *B* after anterior lobe removal. This shows clearly that the influence of the anterior lobe is antagonistic to the action of *B*.

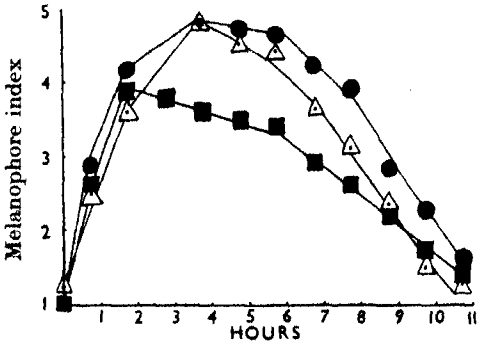

FIG. 7 (c). The responses of the same animals as fig. 7 (*b*), following the removal of the anterior lobe from set ◒ (now written ●) and a dummy operation on set ◪ (now written ■).

(*b*) *Squalus acanthias* L.

The neuro-intermediate lobe alone was removed from two fish. Both the neuro-intermediate and the anterior lobes were removed from three fish. In all of them extreme pallor associated with full contraction of the melanophores resulted irrespective of the background upon which the animals were placed (fig. 8). In only one of these operations was the ventral lobe removed. Sometimes more than one operation was performed on each fish.

The anterior lobe was removed from one specimen which had been white adapted for a number of days. Its melanophores were then fully contracted. The handling necessary to anaesthetize caused considerable expansion of the melanophores. At the finish of the operation they were again fully contracted. Within 5 min. of its return to a white tank it had darkened considerably. In half an hour it was fully dark and maintained this condition. Five days later the ventral lobe was cut out of the roof of the mouth. At the end of this operation the melanophores were still fully expanded and remained so when the fish was returned to a white tank. Subsequent removal of the neuro-intermediate lobe resulted in maximum pallor.

From one fish which had been white-adapted for a number of days the ventral lobe was cut out of the skull. Its melanophore index was then 3·0. The fish darkened on preliminary handling, but the melanophores had again reached a contracted state by the end of the operation. When replaced in a white tank it retained its limit of white adaptation till the next day. It then died.

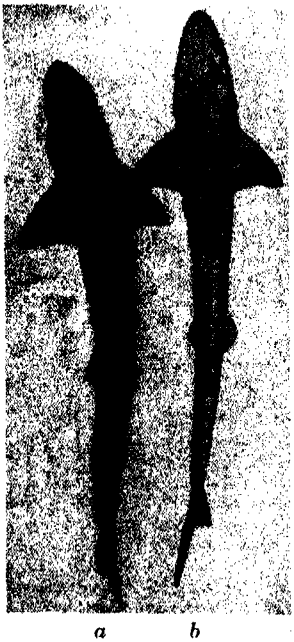

FIG. 8. (*a*) Normal *Squalus* kept on a black background.
(*b*) Hypophysectomized ditto.

From a fish which had been white-adapted for some days and with most of its melanophores fully contracted, skull incisions sufficient to remove the pituitary were made. Despite being kept on a white background for the administration of the anaesthetic the fish darkened considerably and at the end of the operation the melanophore index was 3·0. When replaced in the white tank it slowly regained its full pallor (with some melanophores completely rounded off and others not quite). It took slightly longer to attain full pallor than it had done before operation. Although no parts of the pituitary were removed in this operation, skull incisions sufficient to remove all parts were made with the object of demonstrating that such incisions do not cause sufficient strain on the cranium to damage the optic nerves or interfere with the nerve and blood supply of the hypophysis.

These experiments show:

(1) Removal of the neuro-intermediate lobe results in fully contracted melanophores associated with maximum pallor irrespective of the background. In effect this means that the natural black background response is destroyed.

(2) Removal of the anterior and intermediate lobes together causes the same result as the removal of the latter alone. Full contraction of the melanophores results. In this respect *Squalus* resembles *Scyllium*, *Raia*, *Rhina*, but differs from the amphibian genera *Xenopus* and *Amblystoma*. In the latter the melanophores of completely hypophysectomized individuals are not quite fully contracted.

(3) Removal of the anterior lobe alone causes loss of the white background response.

(4) Removal of the ventral lobe does not seem to interfere with the white background response. This is uncertain owing to the early death of the one experimental animal.

(5) Removal of the ventral lobe does not induce pallor in specimens made dark by removal of the anterior lobe. It is not certain whether removal of the ventral lobe interferes with the normal black background response.

6. SECTION OF PERIPHERAL NERVES

Nerve section has been shown to have no effect on the melanophores of *Scyllium* (Young 1933; Waring 1936; Wykes 1936). So further experiments of this kind were confined to *Squalus*.

Cuts made transverse to the fin rays of a dark fish sometimes caused production of light bands distal to the cuts (fig. 9) as in experiments with *Mustelus* (Parker and Porter 1934). Frequently also there was visible a white area completely encircling the cut (fig. 9*a*). This was similar to the phenomenon illustrated in a figure given by Parker (1936*b*, p. 257, fig. 3). Sometimes no macroscopic paling resulted from fin cuts—an observation which is in agreement with a statement made in a publication by Parker (1936*a*) based on the same fish. Absence of the pale band was not an individual peculiarity of a particular fish, nor of any particular part of the same fish except in so far as cuts made in the fin rays supplying the outer posterior point of the pectoral fin, seemed particularly liable to provoke the reaction. Generally the pale area occupied the full width of the cut and extended to the edge of the fin. On one occasion the pale area consisted simply of a thin but distinct line from one end of the cut to the edge of the fin; on two occasions a pale band, the exact width of the cut, formed. It extended only from half way between the cut and the edge of the fin to the edge.

The blanching is caused by the contraction of both types of melanophores and in most cases the contraction is complete or nearly so. Very commonly, however, small groups of fully expanded melanophores occur in a pale area. Microscopically the white band is very sharply delimited, but there is always a narrow band of 2 or 3 melanophores which show an intermediate stage of contraction between the surrounding dark skin and the pale area. Although cuts frequently produced no macroscopic effect, microscopic examination showed full, or nearly full, contraction of the melanophores in the area delimited by, and distal to, the cut. In some this area might involve a band only 3 or 4 melanophores deep. It must be emphasized that when cuts gave a poor pallor reaction, it was due to the small number of melanophores affected and not due to the partial contraction of a large number. In nearly all, but not quite all, a few melanophores proximal to the cut were affected.

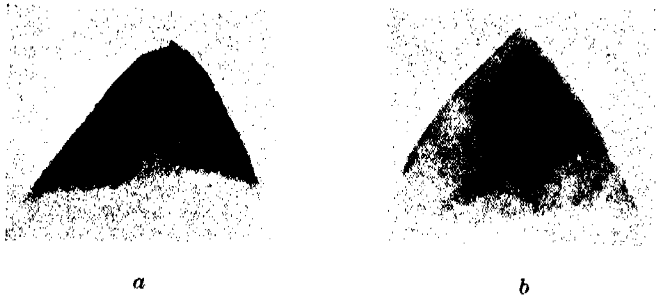

FIG. 9. (*a*) Blanching distal to a cut made in a dark fin. (*b*) Dark area distal to a cut in a pale fin of a hypophysectomized fish.

The pale bands eventually became dark by expansion of melanophores. When the natural death pallor was setting in the former pale areas might pale first. In one fish, hypophysectomized after a pale area had become dark again, the area which was formerly pale, remained dark (fig. 9*b*). The dermal melanophores retained the expanded state, when the rest of the animal had become pale as the result of the operation.

The mode and time of the onset of the blanching, its duration and the mode and time of its obliteration all show considerable variation. The mechanism underlying this blanching which occurs as a result of cutting fin rays is undetermined. It might be due to direct interference with the blood supply to the affected area, to stimulation of the vaso-motor centre, release from central vaso-motor control or to the production of injury currents in post ganglionic vaso-motor neurones undergoing degeneration. Recently Slome and Shaughnessy (1935) have shown that such injury currents can be detected in cut nerves for many days after section.

However carefully the area for cutting is selected some direct interference with the blood supply is inevitable. For the following reasons I do not consider that blanching results from such crude interference with the blood supply:

(i) In all cases at least some blood flow was discernible in the blanched areas.

(ii) The larger blood vessels at least do not follow the lines of the parallel fin rays, and yet the blanched areas are approximately limited by the fin rays.

(iii) In two cases cuts were made deliberately in the path of major vessels and produced no paling effect.

(iv) Cuts made in analogous parts of different fishes may produce blanched areas in one and not in the other.

(v) Similar cuts made in the fins of *Scyllium*, in which the blood supply appears substantially similar, never gave rise to blanched areas.

(vi) In the one investigation which led to the conclusion that cuts similar to those here described had sufficiently interfered with the blood supply to cause blanching (that of Young 1935—on the lamprey), the pale area was always narrower than the length of the cut, and the most affected areas were near to the cut and at the periphery where the blood supply was relatively undisturbed the skin was quite dark. In addition the line between the normal and affected area was not sharp. On the contrary, in the present investigation, the pale area was nearly always as wide as the cut. It was frequently ill-marked in the neighbourhood of the cut, and it was always sharply defined from the normal area.

7. DISCUSSION

Like most other elasmobranchs investigated, *Squalus* shows a well-marked response to varying backgrounds—paling on a white background and darkening on a black background. It has been shown, moreover, in the present investigation, that Parker's failure (1936*a*) to observe these changes can be reasonably attributed to both or either of the following: (*a*) defective sight of his specimens, (*b*) lack of precautions for detecting a strong *primary* response in this species.

It has also been shown in the present investigations that the dark background reaction (expanded melanophores) is undoubtedly dependent on a hormone from the neuro-intermediate lobe of the pituitary. Parker himself (1936*a*) has demonstrated that this darkening hormone (*B*) is carried in the blood stream, and identifies it with the mechanism underlying the dark background reaction. *Squalus* therefore resembles all the other elasmobranchs so far investigated. (Lundstrom and Bard 1932, *Mustelus*; Hogben 1936*a*, *Raia clavata*, *R. maculata*, *R. brachyura* and *Scyllium* sp.; Parker 1937, *Raia erinacea*.) The mechanism underlying the white background reaction (contracted melanophores) is less clear. Excision of the anterior lobe of the pituitary destroys the response in all species examined. The only exception to this statement is Parker's as yet unconfirmed statement concerning *Mustelus*. Extracts and implantation of anterior lobe have not as yet been shown to cause pallor in dark fish, but the evidence from observations on the natural response and from the injection of *B* into partially and fully hypophysectomized fish of the genus *Scyllium* is sufficient to show that the anterior lobe secretes a hormone (*W*) antagonistic to *B*. In effect this means that the mechanism of colour response in elasmobranchs is essentially the same as in amphibia (Hogben and Slome 1936).

If generalized pallor in response to natural stimuli for which the eye is the receptor organ is brought about by the reflex secretion of the anterior lobe, the local pallor seen in Parker's blanched area distal to fin cuts raises a separate issue. There can be no doubt about the facts concerning such blanching. Difference arises only in the interpretation of its significance. As regards its relation to generalized pallor in normal background responses, several possibilities may be advanced. If we reject the view that it is due to direct interference with blood supply, it may arise from the effect of cutting or stimulating (*a*) neurones directly connected with the melanophores, (*b*) neurones connected with peripheral blood vessels.

(*a*) There is no direct evidence for the existence of peripheral nerve supply to the melanophores of elasmobranchs. In view of the immensely reduced reaction times of chromatic behaviour in teleosts the existence of such nerves in bony fishes does not necessarily justify the belief that they also exist in selachians. Assuming that they do exist, several possibilities may be distinguished.

(i) One is that they actively control expansion or contraction of melanophores in the normal rhythm of colour change. If so they may convey stimuli which result in expansion or contraction.

(ii) The other possibility is that they exert a continuous tonic control which maintains the melanophores in a state of partial expansion antagonistic to the action of both the *W* and the *B* hormones.

These possibilities must be severally examined in the light of the known and admitted existence of at least one hormone (*B*). From this point of view two possibilities emerge. One is that the active control is entirely humoral and that the only function of peripheral nerves to the melanophores, if such do indeed exist, is to maintain a state of contraction antagonized by *B* alone (or a state of tone antagonized by *B* and *W*). The other is that the *B*-content or (*B* and *W* content) of the blood is not reflexly affected by the hormone (or hormones) and its action is merely to maintain the melanophores in a state to respond appropriately to central excitations or inhibition through their peripheral nerve supply. The last implies (i) in the preceding paragraph. Parker's own experiments on the transfusion of blood from dark fish and pale fish dismisses the last view that the humoral control is purely static. Hence they also dismiss (i).

In short there is a logical inconsistency in Parker's interpretation of his results. The second alternative (ii), for which there is no evidence, implies that local pallor in the neighbourhood of fin cuts has no direct connexion with the mechanism of reversible generalized pallor.

(*b*) This leaves us with one remaining alternative that the local effect is due to vaso-motor disturbance. Unlike (*a*) discussed above this relies on known data. Local pallor produced by vascular disturbance then gives no clue to the mechanism underlying pallor. Peripheral mixed nerves of *land* vertebrates commonly contain post ganglionic fibres which innervate blood vessels. In selachians (Young 1933) such post ganglionic nerves run along the course of the blood vessels. The effect of cutting fin rays therefore involves two sorts of disturbance to the humoral control of the affected area. One is release from *central* vaso-motor control. The other is *peripheral* stimulation due to injury currents which necessarily arise as the result of section and persist during the process of degeneration. This has been established by recent work of Slome and O'Shaughnessy (1935).

Whether the net result will be local constriction or local vasodilation is impossible to state without direct quantitative experiments on the local blood flow by one of the standard methods. None of these have been used by Parker. The fact that some species react to fin cuts while others do not is not by any means surprising. Whether increased or decreased local blood flow would produce pallor or the reverse is impossible to say, until we know whether the speeds at which *B* and *W* are respectively eliminated are due to the rates at which they are excreted or to the rates at which they are hydrolysed, oxidized or otherwise destroyed by the tissues. From every point of view the class of experiments on which Parker bases his "neurohumoral" interpretation of the co-ordination of colour change in elasmobranchs is therefore unable to yield unequivocal evidence in favour of it.

8. SUMMARY

1. Elasmobranchs with few exceptions pale in illuminated white tanks and darken in illuminated black ones.

2. These changes result from the contraction and expansion of dermal and epidermal melanophores.

3. In semi-darkness an intermediate condition of the melanophores is obtained.

4. Expansion of the melanophores results from the activity of a blood-circulated hormone (*B*) derived from the neuro-intermediate lobe.

5. Removal of the anterior lobe abolishes the white background response.

6. Decreased tolerance to *B* extracts, after removal of the anterior as well as the neuro-intermediate lobe of *Scyllium*, furnishes additional evidence for the secretion of a contracting (*W*) hormone in the anterior lobe.

7. Cuts made transverse to the fin rays of a dark ***Mustelus*** and ***Squalus*** elicit pale areas distal to the cuts. Arguments are advanced for considering that this phenomenon gives no clue to the mechanism underlying normal pallor.

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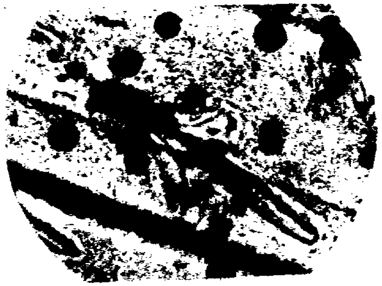
Fig. 10

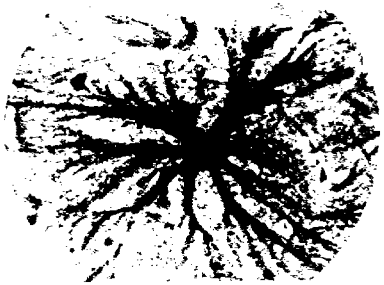
Fig. 11 (*a*)

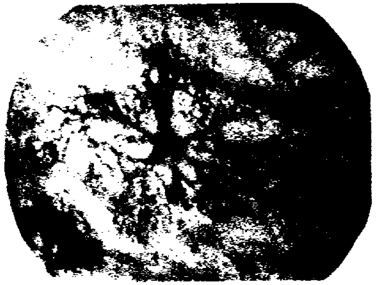
Fig. 11 (*b*)

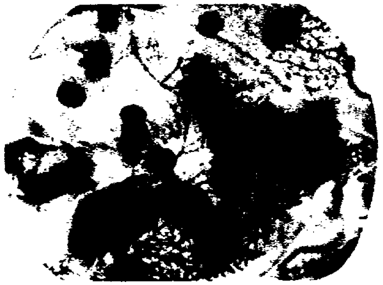
Fig. 13

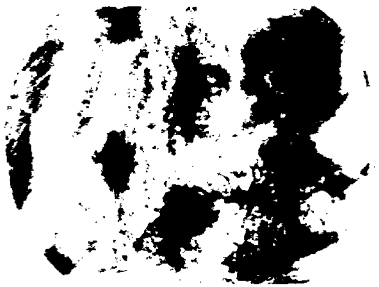
Fig. 12 (*a*)

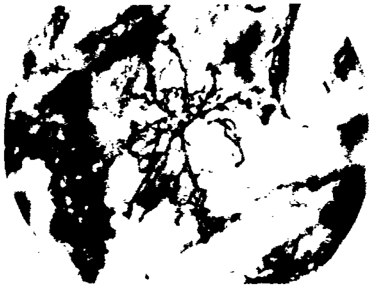
Fig. 12 (*b*)

Squalus acanthias L.

Fig. 10. Dermal and epidermal melanophores fully contracted.

Fig. 11. (*a*) an intermediate } condition of dermal melanophores.
(*b*) expanded }

Fig. 12. (*a*) an intermediate } condition of epidermal melanophores.
(*b*) expanded }

Fig. 13. Static epidermal pigment.

541·135·6:537·742·7

The polarization of a calomel electrode

BY LORD ROTHSCHILD

From the Sub-Department of Experimental Zoology, Cambridge

(*Communicated by J. Gray, F.R.S.—Received* 30 *December* 1937)

INTRODUCTION

The object of this paper is to define, for electro-physiological purposes, the limits within which a calomel electrode is unaffected by electric current passing through it.

The calomel half-cell, though always known as a non-polarizable electrode, is in fact polarizable in the same way as all other electrodes. The only difference between it and a notoriously polarizable electrode such as Pt is in degree.* Since it is incorrect to talk of a calomel half-cell as a non-polarizable electrode, a definition of polarization is necessary: when a metal dips into a solution, there is a p.d. between it and the solution. Provided that (1) the composition of the electrode does not alter, (2) the activities of the components of the solution do not alter, and (3) no current flows through the system, the p.d. between the metal and the solution remains constant. If equilibrium does not exist between the metal and the solution, the p.d. does not remain constant and the metal becomes polarized. Thus polarization may be defined as the production of a thermodynamically irreversible potential at the surface of an electrode.†

The calomel half-cell is so constituted that in spite of current flowing through it, its p.d. remains constant, but this only holds good within certain limits. The polarizability is proportional to the coulomb density at the mercury surface, i.e. the number of amp./cm.^{2} flowing through the electrode multiplied by the time of flow.

The experiments described in this paper establish the time for which an electrical current of known value may flow through a calomel electrode with a known surface area of mercury without that current affecting the normal properties of the electrode. When the current reaches a value such that it does affect the electrode, the latter's resistance changes from its ordinary value of about 30Ω cm.^{2} to an effective value of 40,000Ω cm.^{2}, a fact of some importance in bio-electric measurement and stimulation.

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* Electrochemically, a Pt electrode is a gas electrode using Pt as an inert basis.

† Self-polarization by local action currents is not discussed in this paper.

METHOD

Two calomel electrodes were prepared as in fig. 1. C_1 is the electrode under examination. C_2 is a reference electrode. The half-cells were not

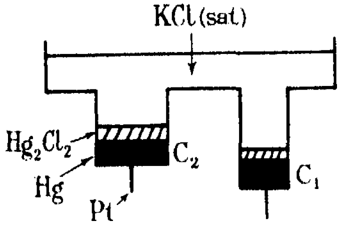

FIG. 1

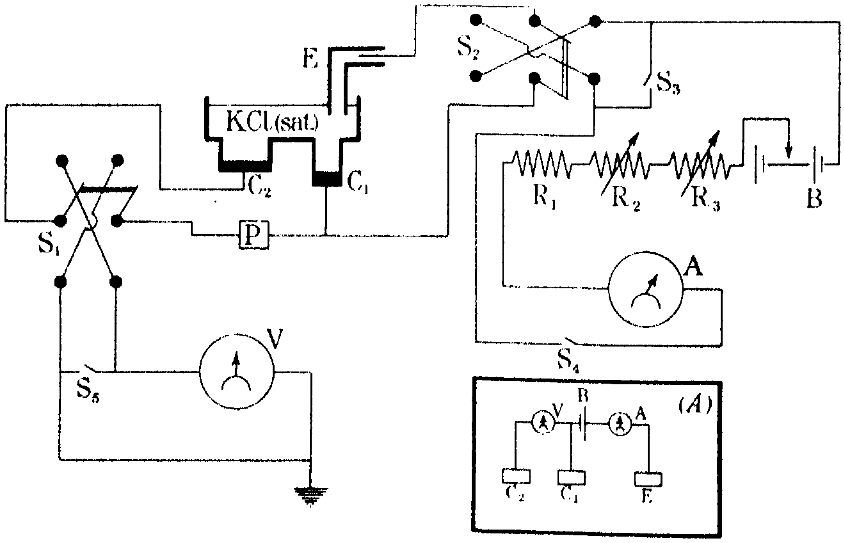

FIG. 2. A, micro-ammeter; B, polarizing battery; C_1, experimental electrode; C_2, reference electrode; E, circuit electrode; P, potentiometer; R_1, 1·0 MΩ; R_2, 5·0 MΩ; R_3, 0·1 MΩ; S_1, S_2, reversing switches; S_3, S_4, S_5, shorting switches; V, thermionic voltmeter.

FIG. 2 (A). Simplified wiring diagram.

prepared in any elaborate way from specially purified components. Ordinary laboratory conditions were reproduced. Redistilled mercury, B.D.H. Hg_2Cl_2 and Kahlbaum or B.D.H. KCl were used. More dilute KCl solutions are frequently used in calomel half-cells for obvious physiological reasons, or bridges from the saturated solutions to more dilute ones. The use of saturated KCl throughout simplifies the experiment and removes a source of error due to evaporation. The effect on polarization of varying the KCl activity may be discussed in a later paper. The electrical connexions are shown in fig. 2. E is a saturated KCl bridge leading to a calomel electrode. Its function is simply to make electrical connexion between the KCl solution above C_1 and the battery B and therefore need not be considered further. When the switch S_4 is closed, an electrical current flows through the resistances R_1, R_2 and R_3,* the ammeter A, the calomel half-cell C_1, the electrode E and back to the battery. Any p.d. between C_1 and C_2 is measured by the voltmeter V. V is a thermionic bridge combined with a potentiometer (Rothschild 1937). As the voltmeter only uses 10^{-13} amp., it has no effect on C_1 and C_2 and this current is considered as 0 amp. in the rest of this paper. The experimental procedure is as follows: S_3 and S_4 are closed. R_2, R_3 and the potential of the battery B are varied until the required current is shown on A. S_3 is then opened and the p.d. between C_1 and C_2 is noted at different intervals of time.

The coulomb density at which marked *anodic* polarization occurs is the limiting factor in using the calomel half-cells as they are much more resistant to cathodic than to anodic polarization (see Appendix). For this reason all experiments refer to anodic polarization.

RESULTS

Fig. 3 illustrates typical relationships between current density, p.d. and time. O is the origin; the current is switched on at O, $T=0$. X represents the conditions existing before current flow begins. Even when no current is flowing, there often is a small p.d. between the two electrodes. It varies between 0·1 and 0·5 mV. All the curves except G, which is a control when no current flows, have been scaled, so that graphically there is no p.d. between the electrodes before the current is switched on. The control G starts at the origin O. The displacement on the p.d. axis of the curves

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* By virtue of their high values, these resistances make the current flowing in the circuit independent of the resistance of C_1 until this reaches a value of approx. 10^4 ohm cm.^{2}. Ordinarily the resistance of C_1 is about 30 ohm cm.^{2}.

D, *E* and *F* at low current densities represents the ohmic potential drop across the electrode due to the flow of current, and at higher current densities, i.e. *A*, *B* and *C*, the beginning of polarization and the ohmic potential drop. If, in fig. 3, polarization does not occur,

$$d(\text{p.d.})/dT = 0. \tag{1}$$

This condition is approximately realized in *D* and is definitely realized in *E* and *F*. At *P*, curves *A*, *B* and *C*, polarization accelerates so much that

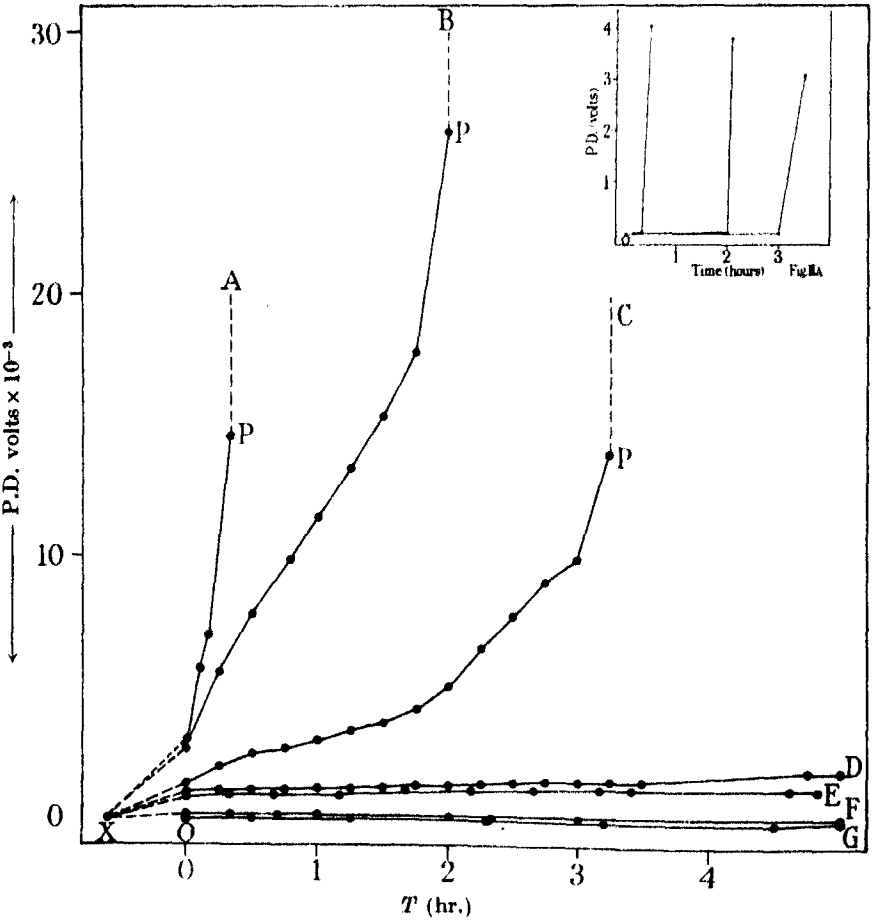

Fig. 3. Electrode (C_1) p.d. plotted against time at various current densities in μA/cm.²: *A*, 108·8; *B*, 81·6; *C*, 54·4; *D*, 27·2; *E*, 13·6; *F*, 6·8; *G*, control, no current flowing through electrode; *P*, time at which quick polarization occurs.

Fig. 3 A. Do. p.d. scale indicates maximum potential reached after polarization.

it cannot be represented on the time or p.d. scale used in fig. 3 (see fig. 3A). Potential differences of 3–4 V may be reached in 7 min.

SUMMARY

The maximum current density which an anodic calomel electrode will sustain over reasonable periods of time (5 hr.) without polarization is $15\mu A/cm.^2$.

THEORETICAL APPENDIX

The so-called non-polarizability of the calomel electrode is primarily due to the buffering effect of the excess solid calomel on the Hg_2^{++} ions in the saturated KCl solution. When the electrode is the anode in an electrical circuit, Cl^- ions migrate towards the mercury surface, and Hg_2^{++} away from it. There are three relationships which must be maintained:

$$\begin{array}{ll} Hg_2Cl_2\ \text{(solid)} \rightleftharpoons & Hg_2^{++} + 2Cl^- \\ & \downarrow\uparrow \\ & Hg\ \text{(metal)} + Hg^{++}, \end{array} \tag{2}$$

$$[Hg_2^{++}]_a\,[Cl^-]_a^2 = K_1, \tag{3}$$

and, if $[Cl^-]_a^2$ becomes small,

$$[Hg_2^{++}]_a\,[OH^-]_a^2 = K_2. \tag{4}$$

In a saturated KCl electrode, at *p*H 5·5–8·0, Hg_2Cl_2 is precipitated initially. At higher current densities this may lead to the removal of Cl^- ions from the solution near the metal surface at a faster rate than they can diffuse in from the bulk phases. Eventually the solution near the metal surface becomes so deficient in Cl^- ions that OH^- ions replace Cl^- ions in the reaction.

According to which of the anions, Cl^- or OH^-, is principally concerned in the formation of the salt, the value of $[Cl^-]_a^2$ or $[OH^-]_a^2$ will vary in equations (3) and (4) above, and thus the activity of Hg_2^{++} will vary. The electrode potential will therefore change, and by definition (see p. 283), the electrode polarizes.*

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* Super-saturation of the solution is prevented by the presence of excess finely divided precipitate.

Two alternative reactions may follow: (1) OH^- ions may react with Hg_2^{++} ions as in the following equation:

$$Hg_2^{++} + 2OH^- \rightleftharpoons Hg_2[OH]_2 \rightarrow Hg_2O[H_2O]. \qquad (5)$$

An oxide film is precipitated as a closely packed and highly resistant film on the mercury surface. (2) OH^- ions may discharge direct on to the oxide surface according to the equation:

$$4OH^- \rightarrow 4\epsilon^- + 2H_2O + O_2 \text{ (gas)}. \qquad (6)$$

The reactions expressed by equation (6) might presumably follow those expressed by equation (5) as the mercury has to become very positive to allow OH^- to discharge. It might be supposed that Cl^- ions could discharge direct on to the Hg surface according to the equation:

$$2Cl^- \rightarrow 2\epsilon^- + Cl_2. \qquad (7)$$

At similar OH^- and Cl^- ion activities, this reaction takes place only at high current densities at a less positive potential than the OH^- discharge; also it will be remembered that there is a deficiency of Cl^- ions while OH^- ions are present. Apart from this there is perhaps some experimental evidence of the deposition of an oxide film. During anodic polarization, a black film is deposited on the mercury surface.*

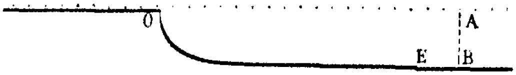

FIG. 4. Rate of decay of polarization p.d. when current is switched off. Time marker 10 c./sec. $AB = 3{\cdot}5$ V. The point E on the record indicates the residual polarization p.d. which persists for some time after the current is switched off (indicated at O).

There appear to be two phases in anodic polarization (see fig. 3 A, B and C), (1) a gradual increase in p.d., and (2) a sudden almost instantaneous increase at P. It may well be that (1) represents the change in Hg_2^{++} activity in solution (see equation (4) *et seq.*), as this p.d. persists when the polarizing current is switched off, which would be expected of a concentration polarization effect (see fig. 4); while (2) represents the deposition of an oxide film. This latter p.d. does not persist when the current is switched off. The rate of decay of the p.d. in phase (2) after the current is switched off has been determined by substituting an electron oscillograph and battery-coupled amplifier for a thermionic bridge as in fig. 2. It will be seen from fig. 4 that the p.d. decay is markedly exponential, indicating that the voltage across the polarized electrode is more due to a capacitative than to a purely resistive impedance. The capacitance of the oxide film cannot be completely evaluated because the resistance and capacitance might not remain constant when the current is switched off. On the assumption that the capacitance and resistance do remain constant, what might be called the "integrated" capacitance of the oxide film has a value of approx. 4μF/cm.².

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* Hg_2O is a black precipitate.

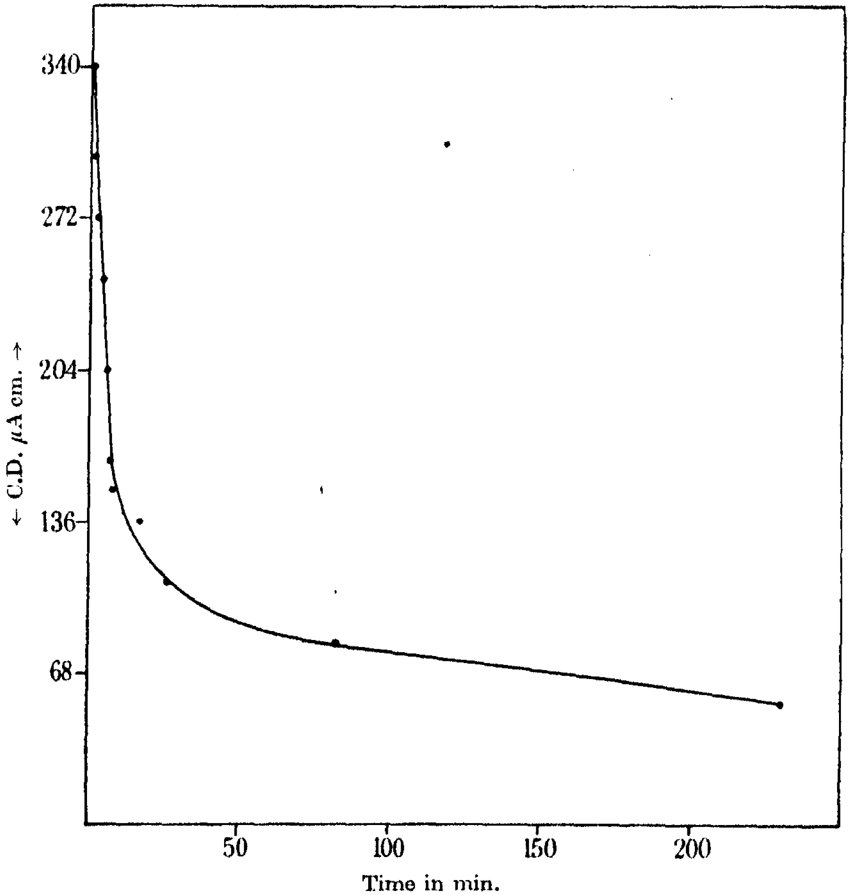

FIG. 5. Polarization time (P) plotted against current density in μA/cm.².

If the time at which phase (2) polarization occurs is plotted against current density as in fig. 5, a very rough approximation to a rectangular hyperbola is obtained. This would confirm that this part of the polarization is a function of the coulomb density at the mercury surface.*

The calomel electrode is more resistant to cathodic than to anodic polarization (see p. 285). The theoretical explanation of this is simple. The anodic non-polarizability of the electrode depends on the ability of the Cl^- ions to diffuse through the closely packed Hg_2Cl_2 layer. The cathodic non-polarizability depends on the dissolution of Hg_2Cl_2. As the calomel is finely divided and thus has a large surface area, dissolution of Hg_2Cl_2 takes place more quickly than diffusion of Cl^-. Therefore the coulomb density necessary to effect cathodic polarization is greater than that required for the anodic process.

I am indebted to Professor E. K. Rideal and to Mr T. P. Hoar for their criticism of the theoretical parts of this paper.

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* Some anomalous results were obtained, particularly at a current density of 54·4 $\mu A/cm.^2$. This is to be expected if elaborate precautions are not taken to obtain absolutely pure components. *p*H regulation by using phosphate buffers saturated with KCl had a stabilizing effect on the polarization time.

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A Discussion
on
New aspects of virus disease

17 *March* 1938

Opening Address

By Dr R. N. SALAMAN, F.R.S.

Recent developments of plant virus research

The last few years have seen a rapid extension of our knowledge of plant virus disease. The field of observation has been extended by the ever-increasing range of plant diseases both of the garden and the glasshouse brought within the purview of the virus worker. It is, however, the fundamental issues of the subject which have been attacked from several angles, to which attention will be directed this afternoon. The new knowledge of the nature of the virus particle, to which Stanley, Bawden, Pirie and Bernal have contributed, has a bearing which, transcending the culture of potato and tobacco, reacts on all biological research and philosophy.

We hope to-day to hear those who speak with authority on the purification and isolation of the virus particle and its chemical and physical structure. Dr Kenneth Smith will tell us of the particle size, and its measurement.

The serological reactions of the plant viruses have assumed increasing importance, and the work of Chester and others has shown how much more may be expected of this line of attack. Not least important is the new knowledge which is emerging as to the relation between the insect vector and the virus itself, on which we hope to hear something from Mrs Watson.

The aspect of the subject I propose to deal with is that of virus strains, their relation to each other and the host plant, and the development of a working hypothesis, to account for the same.

The subject of my study is the X virus of the potato. Six distinct strains of the virus have been isolated in pure plant cultures. Their reactions on tobacco and potato (variety President) are

X^H No visible reaction on tobacco or potato.

X^G A mild mottle on tobacco, and an occasional and transient one on potato.

X^L A tortoise-shell mottle on tobacco and a mild mottle on potato.
X^D The same reaction on tobacco as X^G but a punctate necrotic mottle on potato (Bawden's Foliar necrosis).
Note. Neither X^H, X^G, X^L or X^D produce local lesions.

X^S A necrotic mottle on tobacco and a mild mottle on potato.
X^N A very severe necrotic mottle on tobacco and an interveinal punctate necrosis on potato very similar to that following X^D.
Note. Both X^S and X^N produce local lesions.

It has been shown by the author (Salaman 1933) that if a healthy plant of tobacco, *Datura* or potato be inoculated with either X^H, X^G, X^L or X^D and some 7–10 days subsequently with any of these or with X^S or X^N, no further reaction takes place. In exceptional cases there may be evidence of the presence of the second virus, but then only in a few minute areas. The second more virulent virus is excluded. The basis of the reaction has been referred to under the common adage "First come, first served". This implies the existence of a radicle common to all X virus strains determining their adhesion to the host protoplast, distinct from any which may determine virulence; we may call this "A".

Next we observe that strains X^G, X^L and X^D produce a mottle and no local lesions; X^S and X^N produce the latter and a necrotic mottle. We may interpret this as implying that the first set of strains possess a radicle "M" responsible for mottle production, but that X^S has, in addition, a radicle "N" which induces necroses, local and systemic.

On this hypothesis it is suggested that X^N is furnished with the N but not with the M radicle. This is supported by the fact that no mottle form has been obtained so far by selection or as a direct mutation from X^N. On the other hand, it would be possible to regard the necrotic effects of both X^S and X^N as due to very high quantitative values of M. Certain observations of the behaviour of X^N in the potato varieties King Edward and Eclipse lend some support to this interpretation. In any case the differences between the two sets of strains is wide and unbridged.

The strain X^H, under the conditions of observation in Cambridge, is symptomless; at Rothamsted a very faint mottle has occasionally been observed, hence we must assume that it contains the "M" radicle. It will be observed that there is a clear gradient of virulence from X^H to X^L as measured by their reactions on tobacco or *Datura* which may be explained on a quantitative basis, giving values

$$1M \text{ to } X^H; \quad 3M \text{ to } X^G, \text{ and } X^D; \quad 5M \text{ to } X^L, \text{ and } X^S.$$

On tobacco the reactions of X^D and X^G are identical; on potato, those of X^D and X^N. It is assumed that both X^D and X^N possess a radicle P which has a special affinity for the potato protoplast which the other strains lack.

The composition of the individual strains is further illumined by their behaviour when mixed with non-related viruses and tested out on tobaccos and other host plants.

Mixtures of X strains with the Y and the A virus respectively. In both cases the virulence of reaction, A and Y being constant, varies directly with the virulence of the X strain employed in each mixture, there being always a marked rise between the X^L and the X^S or X^N mixtures. When weak strains of Y are used, the whole range of reactions is reduced in degree but not altered in kind; an explanation on the basis already sketched would seem adequate.

Mixtures of the six X strains with the common, aucuba and masked strains of tobacco mosaic respectively. New features are encountered demonstrating the existence of a more complex situation than in the previous examples. Although none of the three tobacco strains evoke local lesions, yet all the mixtures including that with X^H call forth severe necrotic local lesions which in the cases of X^S and X^N are extensive and very destructive. The strains X^H, X^G, X^D and X^L when mixed with common tobacco mosaic produce an almost identical effect, a medium to severe necrosis accompanied by the normal tobacco mosaic mottle. When X^S and X^N take part, the mottle becomes intensely necrotic, generally killing the early leaves and not infrequently the whole plant. When aucuba mosaic, a more virulent strain, replaces the common tobacco mosaic, the reactions produced are identical with those in the common strain group excepting that the mottle is now a bright yellow on an intense green background. Finally, when the masked type of tobacco mosaic replaces either of the above two strains in the mixture, the host's reactions are indistinguishable from those already described for the mixtures with the common tobacco mosaic virus strain, the only difference being that the symptoms following the masked strain mixtures take longer to mature.

These reactions show that the uniform and medium severe necrotic mottle common to all the mixtures in which X^H, X^G, X^D or X^L are concerned, is not due to the M of the X virus nor, except in part, to the corresponding "M'" factor of tobacco mosaic which we may presume to exist, but to some distinct radicle C which is inert when alone, but which in company with a like radicle in tobacco mosaic C' produces a distinct and uniform type of necrosis which is sufficiently severe to be uninfluenced by "*M*" even when its qualitative value is as high as that in X^L. When, in addition, the radicle "*N*" is superimposed, as it is when X^S or X^N enters the combination, then the reaction reaches a maximum intensity.

The hypothetical radicles can be linked up with certain serological reactions. It has been shown that any one strain is flocculated by the antisera produced from its own or any other strain. The "A" radicle may be identified with the flocculating antigen. Neutralization tests show that there are considerable differences in respect to the extent to which the various strains are neutralized by any one antiserum. The neutralizing antigen may be equated with "*M*" and the differences observed with its quantitative variations. The addition of formalin which destroys the neutralization but leaves the flocculation reaction of the virus intact, supports these identifications.

It is with considerable confidence that one looks to research on plant virus serology for an extension of our knowledge of the structure of the virus particle.

Conversion of strains—mutation. The theory outlined affords an explanation of the occurrence of mutations and conversion of strains.

Change of strain character in the *X* group has been frequently observed, occasionally under controlled conditions, as when strains of X^L which there was reason to believe pure, verted to X^G. Experimental conversion of a pure X^S strain into X^G has been repeatedly affected by passage of the former through local lesions in sugar-beet and a few other plants. These conversions can be ascribed to quantitative changes in the "*M*" accompanied by the loss of the "*N*" radicle. They have not been observed in the X^N strain, which is presumably devoid of "*M*".

All changes spontaneous or experimental have been from a high to a lower virulence. A similar experience has been observed in respect to the *Y* virus. This must not be taken as holding good for all plant viruses.

Whether we are dealing with genuine mutations will probably be always open to doubt. McKinney (1935), however, has made out a good case for the occurrence of such mutations in the virus of tobacco mosiac.

Prophylactic inoculation. Many of our potato varieties in the field are naturally protected against virulent strains of the *X* virus by being themselves infected with a weak one. A notable example is Up-to-Date in which the non-virulent X^H strain is commonly present. Attempts to protect potato plants on these lines against the destructive *Y* virus are in hand. The method has proved successful in the case of tobacco.

K. M. Smith, F.R.S., and W. D. MacClement. *The ultrafiltration of plant viruses*

Elford's technique of ultrafiltration with graded collodion membranes has enabled the particle size of animal viruses to be calculated with considerable accuracy. The application of this technique to the study of plant viruses has, however, been fraught with many difficulties, due mostly to the nature of the viruses and to certain variable factors in their behaviour. From the point of view of size, as determined by ultrafiltration, it appears that the plant viruses may be grouped into two categories, (1) very small viruses, 18–20 mμ in diameter, and (2) medium sized viruses, 100–110 mμ in diameter. There is a third group of viruses which are only filterable through membranes of average pore diameter of 400–700 mμ. The difficulty of filtering viruses of this group may be due either to their large size or, what is more probable, to the fact that the viruses are present in such low concentration that after adsorption to the membrane there is not enough virus left to pass through the finer pore sizes into the filtrate. This fact of low virus content raises the question of concentrating or purifying viruses for filtration, and we have found the rather interesting fact that, after purification or concentration, a virus loses its most intrinsic property, i.e. filterability. That is to say, a purified or precipitated virus will only pass through a membrane of pore size many times larger than that through which the virus will pass when in crude clarified sap. Any process which brings the virus molecules into close proximity causes an apparent aggregation.

We have studied very carefully the filtration end-points of a number of viruses both in crude clarified sap and in various purified and precipitated suspensions. Now, so far as the small viruses are concerned which, by their physical and optical properties, appear to be spherical, we have experienced little difficulty in arriving at an accurate estimate of their particle size which is constant so long as the virus in question is not precipitated. When, however, we attempt to filter those viruses which appear to be rod-shaped, then we encounter a number of anomalies which will be briefly indicated. The viruses in question are *Nicotiana Virus* 1 and its strains (the tobacco mosaic group) and *Solanum Virus* 1 (potato virus *X*). These viruses have been shown by various workers (Bawden and Pirie 1937; Bernal and Fankuchen 1937; Takahashi and Rawlins 1933) to be rod-shaped and not spherical. Considering first the tobacco mosaic virus, we find that this virus has apparently four filtration end-points from which four separate particle sizes can be calculated. There are therefore four sets of conditions which govern these sizes, and only two of these sets of conditions are at present reproducible at will. Briefly, these conditions are as follows: Using a simple cataphoresis apparatus, it is possible by means of an electric current to draw the virus through a membrane. Under such conditions the virus passes a membrane of only 13 mμ average pore diameter. This gives to the virus a calculated particle size of only 4 mμ, unless at this range the ratio of particle size to pore size approaches unity. Secondly, under certain conditions which are not at present known, the virus in crude expressed sap passes a membrane of 50 mμ but not less, this gives a particle diameter of about 22 mμ. Thirdly, under the conditions obtaining with us at the present time the filtration end-point of tobacco mosaic is about 180 mμ giving an average particle diameter of about 106 mμ. Lastly, there is the end-point of about 450 mμ for the virus in the liquid crystalline state. Now what interpretation can be placed on this irregularity of filtration of the tobacco mosaic virus? It raises the question as to what is the ultimate virus unit. It may be suggested that the virus particle which can be drawn by means of the electric current through a membrane of average pore diameter of 13 mμ is the basic unit and that the other sizes are aggregations brought about by varying factors which are not yet known.

A somewhat similar state of affairs exists with *Solanum Virus* 1 (potato virus *X*), though here the smallest particle which we have been able to measure is not less than 100 mμ, while the precipitated virus appears to be in aggregates much larger than those of tobacco mosaic virus.

All attempts to break down the aggregates of these rod-shaped viruses have so far proved unsuccessful. There is, however, a certain amount of evidence that we have succeeded in breaking down the aggregates formed by one of the smaller viruses which is presumably spherical. This virus, *Nicotiana Virus* 11 (tobacco necrosis virus), has a filtration end-point in clarified sap of 45 mμ and a filtration end-point of 145 mμ when purified or precipitated in undialysed sap. If, however, the virus is dialysed against tap water and distilled water either before or after precipitation, then the filtration end-point is the same as for unprecipitated virus, i.e. 45 mμ. In other words, dialysis seems either to prevent aggregation of the virus when precipitated or else to break down the aggregations and allow the virus to pass the filter.

It has been mentioned that tobacco mosaic virus under certain conditions has a filtration end-point of 50 mμ, giving a particle size of about 22 mμ. The various strains of this virus, however, such as aucuba mosaic virus, enation mosaic virus, etc., have never passed a membrane of lower grade than 180 mμ. It is suggested that these viruses either aggregate immediately on extraction from the plant or else are already in a state of aggregation within the plant. Some support is given to this theory by the fact that the end-point for these viruses, precipitated or unprecipitated, dialysed or undialysed, is always the same, i.e. 180 mμ.

F. C. Bawden. *Crystalline and liquid crystalline viruses.*

It has been known for some years that virus-infected plants contain antigens, which are closely associated with the viruses and are not present in healthy plants. Some of these specific antigens have recently been isolated and found to be proteins with large molecular weights. We have isolated the proteins specific to plants infected with three strains of tobacco mosaic virus, cucumber viruses 3 and 4, two strains of potato virus *X* and bushy stunt virus, and the available evidence all indicates that the proteins are the viruses themselves. The particular protein isolated from an infected plant depends solely upon the infecting virus, and it possesses all the characteristic properties of that virus, e.g. resistance to heating, acidity, enzymes, etc. Although the viruses we have isolated differ considerably in their physical and chemical properties they are all nucleo-proteins and can be obtained either in fully or partially crystalline states. The sub-microscope particles contain neither water nor any demonstrable amounts of materials other than protein and nucleic acid, and their constituent units are arranged regularly with the fixed relationship of a crystal lattice.

Solutions of tobacco mosaic virus have a pronounced sheen and other properties indicating the presence of elongated particles. Dilute solutions behave like suspensions of anisotropic rods, readily orientated by streaming, while concentrated solutions are liquid crystalline. Birefringent droplets form in solutions allowed to stand undisturbed; these slowly settle out and originally homogeneous fluids form into two layers, the lower being the more concentrated and liquid crystalline. The layering phenomenon can be used as an index of the purity of preparations, for the formation of a liquid crystalline layer is impeded by impurities, and the purer a preparation is the more dilute it layers and the smaller are the differences in solid content between the two layers. The optical properties also provide a convenient rapid method of testing for the presence of the virus, for nothing has been found in healthy plant sap which shows anisotropy of flow. When solutions are concentrated by high speed centrifugation or by evaporation they form highly birefringent gels. A gel is also produced when the salt content or acidity is raised in a stationary solution. Stirring breaks up this gel, increases the sheen, and precipitates the virus in the form of birefringent, spindle-shaped paracrystals. Paracrystals similar in appearance are also formed when the virus is precipitated with certain histones and protamines.

Although no preparations of tobacco mosaic virus have yet been obtained *in vitro* with a three-dimensional regularity, inclusions are formed in infected plants which are true hexagonal crystals. As the virus content of sap is only 0·2 % and the acidity and salt concentration are insufficient to precipitate the virus, it is probable that the crystals are not pure virus but insoluble virus-host complexes, possibly similar to those obtained experimentally with histones. That the purified virus forms only paracrystals while the virus in the plant forms true crystals may be a result of the increase in size of the virus particles which occurs during purification. Precipitation of the virus causes the small particles to aggregate linearly, and this effect is indicated by the decreased filterability and infectivity and by the increased anistropy of flow of the treated virus. It seems that the small particles in the plant can be arranged regularly into true crystals whereas the greatly elongated particles cannot.

The three strains of tobacco mosaic virus used behave very similarly both in the plant and *in vitro*, but sufficient differences can be detected between their serological and physical properties to show that each is a distinct protein. Cucumber viruses 3 and 4 have a different host range from tobacco mosaic virus, but are related to it serologically, and they behave similarly *in vitro*. No inclusions, either crystalline or amorphous, have been found in plants infected with these viruses, but whether this is due to a difference in the viruses or in the host plants is not known.

Potato virus *X* is not related to tobacco mosaic virus and is much less stable, but its particles are also elongated and preparations of the two viruses have some properties in common. Dilute solutions of virus *X* show anistropy of flow, concentrated ones are liquid crystalline, and the virus forms birefringent gels. When precipitated with acid, ammonium sulphate or histones, however, it does not form paracrystals but an amorphous precipitate, and only amorphous inclusions have been seen in infected plants.

As the viruses all readily sediment in a high speed centrifuge their particles (or molecules) are large, but the size of the smallest particle capable of causing infection is unknown. Their capacity to aggregate linearly into rods makes it impossible to translate their sedimentation constants and filtration end-points into molecular weights. It is possible that differences in the shape or in the degree of aggregation of tobacco mosaic virus and potato virus *X* may be responsible for the present apparently contradictory evidence on their relative sizes, for tobacco mosaic virus is the more readily filterable although it has the greater sedimentation constant.

Bushy stunt virus differs from the others isolated for its particles are not rod-shaped. Purified preparations do not show anistropy of flow, and the gels formed by high speed centrifugation are not birefringent. When precipitated with ammonium sulphate the purified virus crystallizes mainly in the form of rhombic dodecahedra, cubic system crystals which are isotropic. All the viruses yet isolated, therefore, have some degree of crystallinity, but the actual degree differs in the different viruses and, with some viruses, it also varies with the medium containing the virus.

J. D. BERNAL, F.R.S. *The structure of the particles*

It is impossible in the scope of these remarks to give a comprehensive account of the X-ray evidence of the structure and mode of aggregation of the virus proteins, which have been reported in preliminary communications (Bawden, Pirie, Bernal and Fankuchen 1936; Bernal and Fankuchen 1937) and of which a full account is being prepared. More recent work, however, has served to confirm the original conclusions and to throw further light on the internal structure of the particle itself. The X-ray patterns of the "virus" in different states of aggregation can be divided into two of very different character. (The term "virus" will be used to define the protein extracted from a virus-infected plant without prejudice to the question whether it is a virus or not.) We have designated these as inter- and intramolecular patterns respectively, the first occurring at very low angles of diffraction of X-rays and giving information as to the distance between the particles, and the second, at higher angles, corresponding to scattering from inside the individual particle. The intramolecular pattern is, apart from minor variations, independent of the concentration of the virus, from isotropic solutions to dry gels. The intermolecular, on the other hand, while preserving the same general character, varies continuously and quantitatively from one to the other.

We have now new evidence of the independence of these patterns. Dr Fankuchen observed that the relative intensities of the lines of the intermolecular pattern of tobacco mosaic virus were not the same in different photographs, and this was traced to the altogether surprising fact that the specimens prepared by drying a solution of the dimensions $0{\cdot}5 \times 0{\cdot}5 \times 2$ mm. were in fact each a single two-dimensional crystal. The intramolecular pattern given by such a sample examined with the rays parallel to the fibre direction showed a hexagonal pattern of discrete spots.

On the other hand, the intermolecular pattern provided by the same specimen in the same conditions showed completely continuous Debye rings. This can only mean that while the individual long particles are arranged very precisely in hexagonally packed bundles there is no parallelism between the internal arrangements of the particles, each of which gives rise to its scattering pattern independently of the others. This is borne out still more strikingly by further observations on the liquid crystalline solutions.

One specimen of about 25 % dry matter, examined in a thin glass tube, showed singularly good orientation. The lines of the intermolecular pattern were here exceptionally sharp and indicated that even in such a solution the particles are arranged in precise two-dimensional regularity. The intramolecular pattern was even more surprising, showing that the particles were parallel to within less than 2°, and there is a wealth of sharp reflexions which could not be observed on the dried specimens. As the particles in this case are separated by about 150 A of water this scattering must proceed from the particle itself. It is possible to analyse it at any rate approximately as a rotation diagram of a crystal with a hexagonal cell, $a = 88$, $c = 69$ A. There would seem to be only three of such cells in the individual particle, and this gives very nearly the value 153 A observed for the interparticle distance in the dried gel. Such dimensions are very similar to those already observed in a number of proteins (Crowfoot and Riley 1938; Bernal, Fankuchen and Perutz 1938). Taking the density of 1·3 and 9 mol. per cell, which would seem to follow from the symmetry, this would give a molecular weight of 41,000, a not unnatural value for a protein nucleic acid complex. These figures must be taken as provisional, but two definite conclusions can already be drawn; that the separate virus particle has an internal crystalline structure of great regularity, and this structure is analogous to that of other crystalline proteins. There are indications from the strength of the basal reflexions at 23 and 11·5 A that the structure is one composed of definite layers of this thickness. This is also borne out by the slight shrinking in this direction from 11·5 to 11·1 A which occurs on drying.

Two further observations seem to bear this out. One is the existence of true crystals containing the virus in infected plant cells described by Dr Bawden (see discussion above) which would indicate from the existence of pyramid faces a cell of roughly these dimensions, and, secondly, the definitely cubic crystalline character of the bushy stunt virus, X-ray photographs of which show a complete unoriented intramolecular pattern of essentially the same type as that of the tobacco mosaic virus. This would seem to show that the peculiar alisotropic character which first drew attention to the physical properties of the tobacco mosaic virus can be considered rather as a happy accident than as having any fundamental physical or biological significance. It seems probable that the virus particles in the plant consist of flat prismatic protein molecules which attach themselves together very readily to form elongated piles, and it is in this state that they give rise to liquid crystal phenomena and are amenable to X-ray examination in the oriented state.

Finally, a word must be said about the use of the X-ray method for the classification of the different viruses. The viruses we have examined are the three strains of tobacco mosaic virus, two strains of the cucumber, two strains of potato *X* and the bushy stunt virus. We have been able to detect no differences between the different strains of the potato virus and only doubtful ones in the case of the cucumber virus. But with the tobacco virus there are real differences in intensity of the intermolecular pattern which enables the strains to be easily identified. The intramolecular pattern appears to be the same, but good photographs are only available for normal strain. Tobacco and cucumber viruses appear closely related, the general appearance of the intramolecular pattern is the same, but the intermolecular pattern differs, the cucumber being of spacing 4 % less than tobacco and new strong line ($22\bar{4}0$) appearing which is absent or very weak in tobacco. We have so far entirely failed to observe any intermolecular pattern in the potato *X* virus. The intramolecular pattern is definitely different from that of the others, but has approximately the same dimensions and is related to it in an interesting way. The principal axis c seems to be 66–69 A in both cases, but in potato *X* virus it is the second, fourth and sixth orders which are strong instead of the third and sixth as in tobacco. This indicates substantially similar subunits, but a different mode of arrangement. All these viruses show by orientation phenomena the presence of elongated particles. Bushy stunt does not, but its X-ray patterns have not yet been adequately studied. The result of the X-ray classification seems therefore to confirm in every detail that arrived at in serological and clinical observations.

A. S. McFarlane. I would like to direct your attention to some physical properties of one of the animal viruses, viz. that of vaccinia. Sedimentation photographs taken by means of the ultra-violet light absorption technique show diffuse boundaries indicating some variation in the size of the particles. The sedimentation constant is 5000×10^{-13} cm. sec.$^{-1}$ dyne^{-1}. By means of Stokes' law an approximate value for the diameter of the particles (assumed spherical) may be obtained from the sedimentation constant if the density of the virus particles is known. The density of the dry virus as measured with the pyknometer is 1·27, a value which agrees with the low nitrogen content of the virus and with the presence of small amounts of lipoid. When this value is substituted in Stokes' equation the diameter of the smallest virus particles in the suspension is found to be 190 mμ.

In order to determine the density of the virus in the wet state some experiments were performed in which known quantities of sucrose were added to the virus suspension. The virus was then centrifuged down in a laboratory centrifuge and from a determination of the concentration of sucrose in the supernatant the volume occupied by the virus particles can be calculated. Knowing the density of the dried virus it is then possible to calculate the volume of water associated with 1 vol. of dried virus substance. It was found that in contact with a supernatant containing 5% sucrose each volume of dry virus is associated with 1½ vol. of water into which the sucrose does not diffuse. By reducing the sucrose concentration to 2% the volume of water increases to 5 vol. and in 0·5% sucrose each volume of dry virus binds as much as 6½ vol. of water.

At first sight these results suggest that the virus has a semi-permeable envelope and that osmotic considerations determine the amount of water inside the envelope in any particular circumstances. This does not seem to be possible, however, since the mean density of the particle with 6½ vol. of water would be very little greater than the density of the dilute sucrose solution in which it is suspended. A particle with this density and with the known sedimentation constant of vaccinia would necessarily be large enough to be seen in the ordinary microscope. The alternative view that the water is oriented as an "ion atmosphere" in relation to the virus and that in the process of sedimentation this water does not itself sediment is more probably correct. It is supported by electrophoresis experiments which show that the virus particles have an unusually high charge and electrophoretic mobility.

Partition experiments with egg albumin in place of sucrose show that in this medium also the volume of bound water diminishes as the concentration of egg albumin is increased. In M/30 sodium chloride, a medium in which the virus flocculates after 24–48 hr., very little water is bound to the particle and the virus after flocculation appears to be completely dehydrated. Apparently, a water atmosphere is essential to the stability of this large and highly charged particle.

While these observations do not exclude the possibility that the virus is analogous in nature to the smaller bacteria they are more consistent with the conception of a large inert protein particle. It is difficult to visualise metabolic interchanges taking place through a water phase which is not permeable even to sucrose. On the other hand, it must be borne in mind that the experimental facts refer to the virus in artificial circumstances in which no metabolic reactions have yet been demonstrated.

G. M. FINDLAY. *New strains of animal viruses*

Many animal viruses exist in the form of a number of variant strains differing from one another in virulence, in the ease with which they are transmitted by insect vectors, in the type of lesion produced and in antigenicity. The eastern and western strains of equine encephalomyelitis produce almost identical lesions but do not cross immunize: this is also true of the strains of the foot and mouth disease and lymphocytic choriomeningitis viruses. Differences in antigenic structure may not be complete for the swine and the two human types of influenza virus exhibit a certain basic antigenic resemblance.

The Shope fibroma and infectious myxomatosis of rabbits are entirely distinct as regards the type of lesion produced but the fibroma virus immunizes against myxomatosis.

Since variations in antigenic character, in virulence and in the type of lesion produced are by no means rare, the question naturally arises whether new virus entities have arisen in historical time. There is some evidence that swine influenza was not present in the United States of America before the world influenza pandemic of 1918. St Louis encephalitis first appeared in the State of Illinois in 1932, while equine encephalomyelitis and infectious myxomatosis of rabbits almost certainly arose in America after the Spanish conquest when the domestic rabbit was first introduced and the horse was reintroduced into the New World.

Without known changes in environment apparently spontaneous changes may occur in certain animal viruses: similar sudden variations are recorded in the development of yellow mosaic from ordinary tobacco mosaic virus. The rabbit fibroma virus was found by Andrewes and Shope (1936) to change its character and to produce only acute inflammatory lesions.

Under experimental conditions variants can be brought to light by a variety of environmental changes. These virus variants may be classified as:

(*a*) *Variants appearing in a different tissue in a different species*, such as the neurotropic strains of herpes, yellow fever, fowl pest and horse sickness viruses, produced by passage in the brains of mice, a species not usually susceptible to subcutaneous or intraperitoneal injection with these viruses. While pathogenicity for nervous tissues is greatly increased, pathogenicity for the original host by subcutaneous or intraperitoneal inoculation is reduced.

(*b*) *Variants appearing in a different tissue of the same species*, such as neurovaccinia, neurotropic myxoma virus, neurotropic Rift Valley fever virus produced by passage in the brains of mice, neurotropic fowl pest produced in the brains of canaries, neurotropic yellow-fever virus produced in the brains of rhesus monkeys. In cases such as Rift Valley fever in the mouse, yellow fever in the rhesus monkey and fowl pest in the canary where the ordinary strain is so pathogenic that intracerebral inoculation is rapidly followed by death with generalized lesions, neurotropic strains are most readily produced by first injecting immune serum intraperitoneally and a few minutes later the virus intracerebrally.

(*c*) *Variants appearing in the same tissue but in a different species* as in the production of vaccinia from variola in the skin of the calf or rabbit after passage through the skin of the monkey or of the fixed rabies strain from the street virus by passage through the brains of rabbits. In the change from variola to vaccinia, pathogenicity is increased for the rabbit but decreased for primates.

(*d*) *Variants appearing as a result of growth in embryonic tissue* in vitro. The virus of fowl pest and the pantropic strain of yellow fever virus after prolonged passage in a serum-Tyrode medium containing chick or mouse embryo tissue becomes so attenuated that on subcutaneous injection into their original host they are almost non-pathogenic.

(*e*) *Variants appearing as a result of growth on the chorio-allantoic membrane of the developing chick embryo*. The pathogenicity of the influenza virus for animals is decreased by growth in this tissue.

(*f*) *Variants appearing as a result of prolonged growth in mouse carcinoma* in vivo. Prolonged passage of the pantropic strain of yellow fever virus in mouse carcinomata greatly reduces the pathogenicity of the virus for rhesus monkeys.

(*g*) *Variants appearing as a result of alteration in* physical conditions. Armstrong (1929) obtained a strain of vaccinia which grew readily at 37·5° C.

(*h*) *Variants appearing as a result of treatment of one virus by another killed virus*. By inoculating domestic rabbits with a mixture of active fibroma virus and heat-inactivated myxoma virus Berry and Dedrick (1936) obtained not fibromata but typical myxomatosis.

Certain types of virus variants are, to some extent, reversible: other virus variants have been regarded as irreversible. Thus reconversion of vaccinia into smallpox in man has not been recorded, but some degree of reconversion of the fixed to the street virus of rabies was obtained by Nicolau and Kopciowska (1935) by repeated passage of the fixed virus in the sciatic nerves of rabbits. Findlay and Clarke (1935) also obtained reversion of the neurotropic yellow fever virus, after 200 consecutive passages in mouse brain, into the ordinary pantropic strain by repeated intrahepatic passage in rhesus monkeys. Recently it has been found that the neurotropic yellow fever virus, while remaining fixed for mice, has undergone spontaneous reconversion for rhesus monkeys and now produces lesions in the viscera similar to those caused by the ordinary yellow fever virus. Certain of the variations of animal viruses are, it has been suggested (Findlay 1936), similar to the *Dauermodifikationen* described by Jollos (1920) among protozoa, alterations of the hereditary constitution associated with environmental changes but reversible when the environmental stimulus ceases to act. Other virus variants appear to be akin to the mutations of higher animals and plants. The simplest explanation for the appearance of neurotropic variants or of the vaccinial variants of variola and alastrim is that the virus inoculum is either heterogeneous when inoculated or becomes so shortly after inoculation: environmental selection then comes into action.

M. A. WATSON. *Aphis transmission of some plant viruses*

Hyoscyamus virus 3 (Hy 3) is much more readily transmitted by *Myzus persicae* Sulz. if the insects fast for a time before being fed on the source of infection. Even so short a fasting period as 15 min. greatly increases efficiency, and after 1 hr. fasting the number of infections given by single aphides is from seven to nine times that obtained with unstarved insects, in otherwise similar conditions.

This increase in efficiency, however, does not persist if the insects are allowed to feed for long periods on the infected leaves. The optimum feeding time for *M. persicae* on Hy 3 infected plants is only 2 min., and after 1 hr. the number of infections obtained is the same as for unstarved aphides.

This effect is probably caused by the virus coming into contact with some substance which inactivates it and is produced in greater quantities when the aphid is feeding than when it is not. The association of virus inactivation with aphid feeding suggests that it might be caused by a digestive enzyme, particularly as the infectivity of Hy 3 preparations is immediately and quantitatively reduced by mixing with solutions of Merck's pancreatin. None of the results so far obtained disagree with this hypothesis.

It is paradoxical that such results, and all other results so far obtained, would suggest that the conditions of aphis transmission are against the less stable viruses of low infectivity, which are in fact transmitted, and in favour of the highly infectious, highly resistant viruses, such as the tobacco mosaic group, for which no vector has been found. No explanation for this curious anomaly can be put forward. Tests have been made to determine whether the starving effect could be demonstrated for other viruses and vectors, for if it were in fact attributable to some common function such as the production of proteolytic enzymes, any virus with similar physical properties to Hy 3, when transmitted by insects similar to *M. persicae*, should be similarly affected.

The viruses used in these tests were: Two strains of Hy 3 virus, one of which, an almost symptomless variant, has not previously been described, one strain of potato virus *Y*, and two strains of cucumber mosaic virus, namely "Johnson's cucumber mosaic" which gives green symptoms in tobacco plants, and Price's No. 1 strain, which gives intense chlorosis. Both *M. persicae* and *M. circumflexus* were found to be successful, in varying degrees, in the transmission of all these viruses.

For the Hy 3 viruses and potato virus *Y*, *M. persicae* was found to be a much more efficient vector than *M. circumflexus*, though both responded proportionally to the preliminary fasting treatments. With the cucumber viruses neither insect was as successful as with any virus in the first group, but *M. circumflexus* was at least as good, and probably a better vector than *M. persicae*. There was also a good response to preliminary starving, but with *M. persicae* this differed from that observed for the other viruses, in that the optimum feeding time on the infected plant was longer.

There appeared to be some relationship between the infectivity of the viruses as determined by the starch lesion count method, and their transmissibility by the vectors, but this was only observed within the two groups of viruses, and not between them. For instance Hy 3 virus gives about twice as many lesions as potato virus *Y* when inoculated under identical conditions, and about twice as many aphid infections are obtained with it: the weak Hy 3 strain gives very few lesions and its transmissibility by insects is equivalently low. Similarly, of the two strains of cucumber virus the yellow strain, which gives a lower lesion count than the green, is correspondingly difficult to transmit with both species of aphid. On the other hand, the green strain of cucumber mosaic virus gives about the same lesion count as potato virus *Y*, though its transmissibility by *M. persicae* is only about one-tenth.

It seems that such methods might be of further use in determining differences in the relationships between strains of viruses, and between groups of similar viruses which do not show strain relationships.

P. A. MURPHY. *Application of new knowledge of viruses to disease control*

Plant virus workers may be congratulated on the recent notable advances in knowledge of virus composition. Only a little while ago the agent was confidently assumed to be a form of life of some sort, that is a parasitic organism, but now a new conception emerges, that of a parasitic substance. This in Stanley's (1937) language may be considered to auto-catalyse itself when combined with the living protoplasm of a congenial host, and apparently also of its vector in some cases.

No increase of a virus outside a living cell has yet been established. I am not directly concerned with the nature of viruses, but every fundamental advance furnishes the applied worker with a new weapon.

The discovery of strains of the same virus such as those of Solanum Virus 1 by Salaman (1933) and others, and those of Sol. V. 2 and Sol. V. 3 (Murphy and Loughnane 1936), and the fact that strains of the same virus cross-immunize against each other, is of much practical importance. This is particularly so in the case of Solanum Virus 1 (virus *X*), which is abundant everywhere and has defied control up to the present. This consists of many strains, and it has been plausibly suggested that the reason why an outstanding variety of potato like Up-to-Date resists degeneration so long is that the whole clone became infected by chance with a mild strain of Sol. V. 1, which protects it from more severe strains of this virus. The question arises at once for the seed-potato grower—is he to rely on protective inoculation or to produce virus free stocks?

Before answering this it is necessary to consider the different types of viruses which occur, as for example in potatoes. The transmission of the important *X*-group of viruses of this crop has now been discovered, as well as that of Sol. V. 4 (top necrosis) and Sol. V. 8 (tuber blotch) (Loughnane and Murphy 1938*b*). This takes place by contact of the leaves, as in the wind, and is no doubt effected through broken hairs. Some proof of this is found in the fact that a healthy plant placed in contact with a plant containing two viruses, to both of which the first plant is susceptible, sometimes becomes infected with only one member of the combination, as shown by Loughnane and Murphy (1938*a*, *b*). This probably indicates that the component viruses are not equally distributed in the original plant, and the probability is that infection took place from and to a single broken hair.

It is now possible to divide the potato mosaic viruses into three groups—the contact viruses of which Sol. V. 1 is the type; the aphidine viruses such as Sol. V. 2 and Sol. V. 3 and the leaf roll virus; and those transmissible by both means, such as Sol. V. 8 and probably Sol. V. 2. Neglecting leaf roll which is a special problem, the principal of these viruses and the ones responsible for nearly all degeneration are Sol. V. 1, Sol. V. 2, and Sol. V. 3.

Natural protection against a virus takes three forms, as follows:

A. Intolerance, as a result of which the plant recovers completely without developing immunity, this being the only case in which the plant becomes virus-free.

B. Tolerance, as a result of which the plant carries virulent forms of a virus without notable symptoms or ill effects.

C. Protective inoculation, as it may be termed, or the prior presence of a weak virus strain which protects the plant from other related forms, which would otherwise be serious. In this case the plant continues to harbour the weak virus indefinitely.

Protection against Sol. V. 1 is effected by methods (A) and (C); against Sol. V. 3 by methods (A) and (B); and against Sol. V. 2 by method (B).

The control of Sol. V. 1 in the field has proved the most difficult problem of all, and in fact has been insoluble up to recently. This is the characteristic virus of the seed-producing areas, and is the main cause of degeneration there, the aphidine viruses being scarcer, especially Sol. V. 2.

The fact that some of the outstanding varieties are naturally permeated by a weak strain of Sol. V. 1 would appear to indicate that protective inoculation might provide the key. Further experience must decide this, but it may be pointed out that the presence of any virus is potentially dangerous, either by combining with another to reproduce a more serious disease, or because of the possible danger of mutation to a more lethal form. The complete absence of all viruses, including Sol. V. 1, would be a higher ideal, and recent work indicates that this is not unattainable. Seed potatoes have now been grown in the open for six years in Donegal in a virus-free condition, and production on a commercial scale does not appear impossible (Clinch, Loughnane and Murphy 1938). Actually Sol. V. 1 is one of the easiest instead of the most difficult virus to control, for moderate isolation is sufficient to exclude it, and it appears likely that direct contact of the leaves is the principal method of spread, though mechanical transmission over a distance has not yet been excluded. There is no evidence of a natural reservoir of infection in wild plants.

W. J. Elford. The first point I should like to make concerns the application of ultrafiltration methods in estimating the sizes of plant viruses. It is perfectly clear from Dr Kenneth Smith's work that there are peculiar difficulties to be encountered due to such factors as surface adsorption, aggregation, and low virulence of the plant extracts. Further, in instances where the particle departs appreciably from the spherical shape mechanical blocking of the pores will doubtless be incurred and conditions most favourable for filtration will be much more critical than in the case of spherical particles. The limiting porosity will be in relation to the width of the particle (cf. filtration of spirochaetes, Hindle and Elford 1933). When using the gradocol series of membranes and the relationship I have found for deducing the probable particle size from the limiting pore size (Elford 1933) it is imperative that the method of calibrating the membranes—rate of flow of water—and the experimental filtration conditions—namely those most favourable for filtration with adsorption at a minimum—apply as in the standardizing experiments. The revelation by Dr Bawden that certain plant viruses may exist in the crystalline state naturally in infected tissue suggests that the conditions prevailing in the juices of such tissues may not be those best suited for filtration. It is to be expected, of course, that some elementary virus particles will also be present but as a whole the conditions are those favouring aggregation and crystallization. It seems probable therefore, and in view of the results obtained using Hartley's broth at pH 7·6 with proteins and animal viruses, together with the findings of Thornberry (1935) with numerous plant viruses, that an extended investigation of the influence of the reaction and constitution of the medium on the state of dispersion and adsorbability of plant viruses may reveal conditions that are generally more favourable for filtration than are those constituted in plant juice.

Secondly I should like to supplement Dr MacFarlane's remarks on the apparent density of vaccinia virus. The possibility of obtaining concentrated purified suspensions of vaccinia elementary bodies and also of certain of the largest bacteriophages has permitted the direct measurement of the displacement of the boundary in the inverted capillary tube method of studying centrifugal sedimentation (Elford 1936). The boundaries are sufficiently stable to be examined after withdrawing the tubes, the capillary column of virus or phage being readily observed when suitably illuminated, viz. vaccinia by oblique illumination and the phages by illumination at right angles to the direction of observation, i.e. seen in terms of scattered light. Visual measurements may be made against a glass scale, or alternatively the capillary may be photographed. Fairly accurate measurements are in this way possible so that the velocities of sedimentation in media of densities and viscosities only slightly different from normal physiological conditions can be ascertained. Then the value of the density of the particle can be calculated from two simultaneous Stokes' equations. The density of vaccinia virus found in this way is 1·17 ± 0·02. Similarly, that of two of the largest bacteriophages—*Staph.* "K" and *Coli* "WLL"—is 1·22 ± 0·02.

The hypothetical structure suggested by Dr MacFarlane for the vaccinia virus particle, namely as a kernel of protein surrounded by a shell of water, requires some further consideration in the light of all available evidence. The ultraviolet light photographs by Barnard (1932) strongly suggest a coccoid form for the virus, i.e. the reverse order of things—a protein shell with the water wholly or in part within.

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The maternal effects on growth and conformation in Shire horse-Shetland pony crosses

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[Plates 14, 15]

Introduction

The relative importance of heredity and environment in determining the size, development, and ultimate proportions of animals has attracted some scientific study and discussion, but little planned experimentation has been attempted with mammals. In the mammal the early and formative stages of development take place in the uterus, where the environment is remarkably constant, and it is not easy to subject the foetus to varying conditions and at the same time not overstep the normal physiological limits. Previous work on the rabbit (Hammond 1934 *a*; Wishart and Hammond 1933), however, has shown that the size of young at birth is approximately inversely proportional to the number in the litter, and it appears probable that some internal secretion or metabolic product of the mother forms a limiting factor in the growth of the embryo. In a monotocous species this effect does not appear, although exceptional twinning or polytocous births reveal its existence, the young at birth being regularly smaller. That the absolute amount of this hypothetical growth-promoting substance may be related in some way with the size of the mother is suggested by the figures of Gregory (1930), which show that the average number of ova of rabbits increases with the average size of the strain. In a monotocous species therefore the size of the mother might determine the size of the offspring at birth. This is also suggested by the results of reciprocal crosses between donkey and horse. The mule (donkey ♂ × horse ♀) is larger than the hinny (horse ♂ × donkey ♀) (Plumb 1916). Since, however, in a species cross the objection may be raised that abnormal genetic segregation might affect the result, we selected for our experiment two breeds of the same species, but of markedly different size, namely, the

Shire horse and the Shetland pony, and reciprocal crosses were made to see how far the size of the mother would affect the size of the offspring. No genetical analysis of size inheritance in horses has yet been attempted, and therefore it may be said that the genetic potentialities of our material were unknown. In the absence of evidence to the contrary, however, we considered it justifiable to assume, on the basis of much genetical experimentation on size inheritance, that a large number of factors would be involved, that segregation and recombination would be at random, and that the offspring of the two breeds would be genetically intermediate, irrespective of which way the cross was made. Any difference between the offspring of the reciprocal crosses would therefore be due not to chromosomal differences but to differences in the environment brought about by difference in the size of the mother. In other words we would have a controlled experiment in which "Mother-size" was the only or predominating variable.

The horse is particularly well adapted for studying the effects of maternal influence on the offspring, since the duration of pregnancy is relatively long (11 months) and the foal is born in an advanced stage of development, particularly with regard to the skeleton (see Meek 1901). Consequently, the effects of maternal influence acting over a long and formative period should be well marked.

The specific problems which we set out to investigate were:

(1) Would the offspring at birth be intermediate in size between the normal offspring of the parent breeds? If this were to be so the foetus in the small mother would certainly be too large and would either cause death of the mother or very difficult parturition or possibly premature abortion, while in the large mother the foal would be very much smaller at birth than normal for the breed.

(2) Would, on the other hand, the size of the mother by virtue of the growth-promoting substance available exert a controlling influence on the size of the foetus, so that in the case of the small mother the foetus would be limited in growth to such a size as she would normally give birth to, while in the case of the large mother the foetus would develop up to the size normal to the large mother?

(3) If a difference in size at birth were established, would this affect the subsequent growth and permanent proportions of the body?

(4) If permanent differences in adult life were established, would these differences be transmitted to subsequent generations?

Naturally a complete answer to the last question cannot be given for several years but we now have evidence to present on the first three.

The experiments relate to several problems of scientific interest, such as the relative importance of heredity and environment, the effect of maternal nutrition on the growth and development of the offspring and the inheritance of "acquired characters". From an agricultural standpoint the experiments bear on the practical problems of making crosses between breeds of different size, on grading up stock for size, and on the influence of early nutrition on subsequent growth and proportions.

EXPERIMENTAL PROCEDURE

The small stud of Shetland ponies kept at the Animal Research Station, Cambridge, was used for this experiment. We obtained the use for breeding of a Shire mare from the University Farm close at hand. Pedigree Shire stallions were only available at some distance from Cambridge, but this difficulty was overcome by the collection of semen at the stud and its transportation to Cambridge. Owing to the difference in size of the parents natural coitus was of course impossible and artificial insemination had to be used. The methods were those developed in Russia and by ourselves, and previously described (see Imp. Bureau Animal Genetics 1933; Walton 1938). The mares at the Animal Research Station were tried daily with a sterile (vasectomized) stallion to determine oestrus. When one or more of the Shetland mares were on heat arrangements were made by telephone to collect Shire semen at the distant stud. Usually an oestrous mare (Shire) was available at the stud, and collection was made by means of the artificial vagina. The semen was brought back to Cambridge as soon as possible and the Shetland mares inseminated. The usual time between collection and insemination was about two hours.

When the Shire mare on the University Farm was in oestrus she was brought to the Research Station. Semen was collected from the Shetland stallion by means of the artificial vagina, using one of the Shetland mares as a mount, and the Shire mare was inseminated with the fresh semen. The percentage of successful inseminations was low (about 20%). This, however, may have been due to inexperience with the method, or merely chance, and does not necessarily imply any incompatibility between the parents.

Details of the successful inseminations are shown in Table I.

PREGNANCY AND PARTURITION

Pregnancy was first recognized in the inseminated mares by the absence of heat and was confirmed by means of the mucus test (see Hammond

1934 *b*). During pregnancy the Shire mare was used on farm work until shortly before foaling and was kept in good working condition. The Shetland mares were run out in grass paddocks with plenty of wild white clover. They were only fed hay during the winter. They did no work and were in very good condition. An open shed gave shelter when wanted. The Shetland pony is very hardy and accustomed to the rigors of the open hillside or moor. Foaling takes place naturally in the open. For this reason no special precautions were taken on approach of parturition except to provide separate paddocks for the foaling mares.

In 1934 the Shire mare ("Daisy") in foal to the Shetland stallion, and the Shetland mare (S 1) in foal to the Shire stallion, foaled during the night without assistance, and the foals were found running with the mothers in the morning. In 1936 the two Shetland mares (S 1 and S 5) which were in foal to Shire stallions were given the same treatment, but the foals were found dead shortly after parturition. As will be seen from Table I, the weights of these foals were perfectly normal for the weights of the dams. There was no sign of dystokia. The placentas and foetuses were perfectly normal in appearance. The cause of death was accidental suffocation by the foetal membranes, as birth occurred during the night when they were not under observation. Had there been any difficulty at birth the mares would have required help.

In 1936 the Shire mare "Daisy" produced a second cross-bred foal. In addition to the cross-bred animals we had pure Shetland foals with which to make comparison of weights and growth rates. From birth onwards weighings, measurements and photographs of the foals were made periodically. The data are recorded in Tables I, II and III, and photographs of the parents and offspring, all brought to the same scale, are shown in Plates 14 and 15.

The principal features shown in Table I will be briefly noted here but a full discussion will be given later.

Duration of pregnancy. The average duration of pregnancy in the horse is about 340 days, but varies considerably with the time of year the foal is born and other circumstances (see Hammond 1934 *b*). As the date of service of the pure Shires was not recorded, the average duration for the species is given in brackets. The table shows that the duration of pregnancy varied considerably, but that there is no obvious relationship to the size of dam nor size of foetus. There is no significant difference between groups.

Weight of dam. The size of Shetland ponies varies considerably. Much higher prices are paid for the smallest animals. The ones we were able to

TABLE I. DATA RELATING TO PARTURITION

	Pure Shetland				Shetland-Shire crosses					Pure Shire	
					From Shetland dam			From Shire dam			
No. and sex of foals	S 5♀	S 6♀	S 7♂	S 50♀	D 2♀	D 4♀	D 5♀	D 1♀	D 3♀	Sh 1	Sh 2
Dam	S 4	S 3	S 5	S 1	S 1	S 1	S 5	Daisy	Daisy	Rosie	Pedigree mare
Sire	S W	S W	S W	S W	Bower Romancer	Bower Duke	Bower Duke	S W	S W	Ouse Bridge Albert	Pedigree Stallion
Date of insemination	*	17. viii. 33	22. v. 36	12. vii. 36	24. vii. 33	9. viii. 35	27. v. 35	30. vi. 33	16. v. 35	*	*
Date of birth	15. iii. 33	15. vii. 34	30. iv. 37	31. v. 37	6. vi. 34	28. vi. 36	10. v. 36	30. v. 34	23. iv. 36	9. v. 36	18. v. 36
Duration of pregnancy (days)	—†	334	343	323	319	337	349	336	343	(340)	
Wt. of dam (kg.)	207	142	185	229	229	229	185	797	797	(800)	(800)
Birth wt. of foal (kg.)	19·5	18·2	22·2	18·6	17·2	13·6	22·7	53·5	45·3	59·4	82·6
Wt. of placenta: Chorion	—†	1·056	1·553	1·030	0·910	1·210	1·672	3·860	2·790	3·810	5·098
Amnion	—†	0·421	0·464	0·297	0·412	0·213	0·580	1·180	0·685	1·027	1·805
Chord	—†	0·090	0·077	0·080	0·088	0·259	0·107	0·257	0·203	0·184	0·405
Total	1·513	1·567	2·094	1·407	1·410	1·682	2·259	5·297	3·678	5·021	6·308
Wt. of foal and placenta	21·0	19·8	24·3	20·0	18·6	15·3	25·0	58·8	49·0	64·4	88·9
$\frac{\text{Wt. of placenta}}{\text{Wt. of foal and placenta}} \times 100$	7·2	7·9	8·6	7·0	7·6	11·0	8·8	9·0	7·5	7·8	7·1
	As percentage of weight of dam										
Birth wt. of foal	9·4	12·8	12·0	8·1	7·5	6·0	12·2	6·7	5·7	7·4	10·2
Foetal membranes	0·7	1·1	1·1	0·6	0·6	0·7	1·2	0·7	0·5	0·6	0·8
Foal and membranes	10·2	13·9	13·1	8·7	8·1	6·7	13·4	7·4	6·2	8·0	11·0
	Group means										
Wt. of dam	190·7				214·1			797·0		(800)	
Wt. of foal	19·6				17·8			49·4		71·0	
$\frac{\text{Wt. of foal}}{\text{Wt. of dam}} \times 100$	10·3				8·3			6·2		8·9	

* From normal matings. † Not recorded.

obtain for experiment were about average size and not the smallest procurable, yet even so there is a very great difference in size between a pony weighing 200 kg. and a horse weighing 800 kg. Exact weights of the Shire mares were not available, but the mare used in the experiment weighed 797 kg., and 800 kg. or 16 cwt. is recognized as about average weight.

Weight of foals. It will be seen that the weights of the cross-bred foals out of the Shetland dams are not significantly different from the weights of pure Shetland foals. The weights of the reciprocal cross are smaller than the weights of the pure Shire, but obviously there are not sufficient data to show whether this is significant, although it may be so. Of the pure Shires, one was small for the breed and the other large. Mr A. G. Holland, Secretary of the Shire Horse Society, informs us that the weights of good pure-bred Shire foals at birth are between 68 and 77 kg. (150 and 170 lb.). It is obvious, however, that in each reciprocal cross there has been very complete regulation of the size of the foal, the foals in the small mothers being limited in size to that of Shetland offspring and those in the large mother growing unchecked, although perhaps not quite reaching the full size of a Shire foal. When the weights of the foals relative to the weights of the dams are compared it will be seen that the percentage weights are not significantly different, except possibly that the cross-breds from the Shire dam are below those of the other groups. The weights of all foals are about 8 % of the weights of the dams.

Weights of placentas. The placenta of the horse is non-decidual so that the afterbirth represents only foetal material. Up to birth the placenta is an integral part of the whole animal of which it constitutes about 8 %. Since the placenta is an organ of foetal nutrition and may also have an endocrine function, its size may be an important correlate of foetal size and development.

POSTNATAL GROWTH IN WEIGHT

All the data for postnatal growth are given in Table II. It will be seen that within the two groups for which we have more than one representative there is fairly close agreement. For the purpose of our analysis, however, we shall confine attention to the three animals—S 6, D 1 and D 2—representing respectively pure Shetland, Shetland-Shire cross from Shire dam, and Shetland-Shire cross from Shetland dam. All three foals are of the same sex (female) and were born in the same year, within a period of about

TABLE II. WEIGHTS AND GROWTH RATES OF FOALS (ALL FEMALES)

S 50 Pure Shetland

Age in months (4 weeks)	Weight in kg.	Growth rate per month	Relative growth rate % per month
0	18·2	24·4	85·1
1	42·6	11·0	23·0
2	53·6	15·8	25·8
3	69·4	10·9	15·8
4	81·3	7·6	8·9
5	88·9	4·6	5·1
6	93·5		

S 6 Pure Shetland

Age in months (4 weeks)	Weight in kg.	Growth rate per month	Relative growth rate % per month
0	18·1	18·2	69·6
1	36·3	12·3	29·2
2	48·6	10·9	20·2
3	59·5	17·7	26·0
4	77·2	9·5	11·6
5	86·7	2·3	2·6
6	89·0	3·6	4·0
7	92·6		

Calendar months	Weight in kg.	Growth rate per month	Relative growth rate % per month
0	18·1	11·3	29·5
5·4	89·0	6·6	6·1
11·0	125·8	6·1	4·5
16·6	160·7	− 2·0	− 1·3
20·7	152·5	11·9	6·4
27·0	227·2	− 3·3	− 1·5
31·8	211·3	8·2	3·6
37·1	255·0		

D 2 Cross-bred from Shetland dam

Age in months (4 weeks)	Weight in kg.	Growth rate per month	Relative growth rate % per month
0	17·2	22·7	84·2
1	39·9	14·5	31·0
2	54·5	17·7	28·2
3	72·1	9·2	12·0
4	81·3	9·5	11·1
5	90·8	10·4	10·9
6	101·2	11·4	10·7
7	112·6		

Calendar months	Weight in kg.	Growth rate per month	Relative growth rate % per month
0	17·2	15·3	32·2
5·5	101·2	7·3	6·1
11·1	142·1	13·2	7·6
16·4	212·0	− 3·7	− 1·7
22·0	191·1	16·9	7·0
28·3	297·8	− 4·8	− 1·7
33·1	274·9	10·3	3·4
38·4	329·4		

D 1 Cross-bred from Shire dam

Age in months (4 weeks)	Weight in kg.	Growth rate per month	Relative growth rate % per month
0	53·5	43·6	59·6
1	97·1	27·7	15·1
2	124·8	23·1	27·0
3	147·9	26·4	16·4
4	174·3	− 0·9	− 0·5
5	173·4	16·8	9·3
6	190·2	23·2	11·5
7	213·4		

Calendar months	Weight in kg.	Growth rate per month	Relative growth rate % per month
0	53·5	24·9	23·1
5·5	190·2	9·2	4·5
11·0	240·6	10·6	3·9
16·6	300·1	− 5·4	− 1·9
22·2	269·7	24·0	7·1
28·5	421·0	− 8·6	− 2·2
33·3	379·5	14·8	3·6
38·6	458·0		

D 3 Cross-bred from Shire dam

Age in months (4 weeks)	Weight in kg.	Growth rate per month	Relative growth rate % per month
0	45·7	33·2	54·6
1	78·9	35·3	37·0
2	114·2	21·8	17·5
3	136·0	12·7	8·9
4	148·7	− 0·5	− 0·3
5	148·2	62·6	35·2
6	210·8	− 4·6	− 2·2
7	206·2		

Calendar months	Weight in kg.	Growth rate per month	Relative growth rate % per month
0	45·7	28·0	26·4
5·8	210·8	9·7	4·8
10·8	268·0	11·3	3·8
15·8	324·4		

6 weeks. They were on pasture together with their mothers until weaned at 4 months. During the rest of their lives they have been grazed together in the open, getting only grass and in winter some hay. Although losing weight during winter they have never fallen out of condition and have been perfectly healthy. Each foal was weighed at 4-week intervals from birth up to 28 weeks, i.e. over the period of most rapid growth. The foals were then weighed when about 11 calendar months old and at approximately 6 months interval after that. The biennial weighings were so arranged that one fell about August and the other about March in order that full effect might be given to the differences in nutrition during spring and early summer when there is a plentiful supply of grass, as contrasted with autumn and winter when the nutritive value of the pasture is low and grazing has to be supplemented with hay.

Growth curves of absolute weight (kg.), monthly gains (kg./month) and percentage monthly increments $[100 \log_e 2 - \log_e 1]$ are shown in figs. 1, 2 and 3 respectively for the early period of growth. From these graphs it will be seen that D 1, the cross-bred from the large mother, grew rapidly, putting on large monthly gains. This rapid growth may be due in part to the plentiful supply of milk from the large mother. We have no actual measurement of the milk flow, but we assume that it would be sufficient to maintain a normal Shire foal. That the growth of the foal was to some extent dependent upon the milk supply of the dam is shown by the rapid drop in the growth curve which follows weaning at 4 months. Unfortunately, we have no actual measurements of Shire foals with which to compare the growth of D 1, but even in this early period it was apparent that the foal was growing less rapidly than a normal Shire. Fig. 4 gives some data on Shire growth, and from this curve it is obvious that D 1 diverges from the normal Shire curve at an early stage. In contrast to D 1 the cross-bred from the small mother grew less rapidly, and this was possibly due to some extent to the limitation in the milk supply from the small mother. The foal did, however, grow more rapidly than S 6 (pure Shetland), although it was receiving approximately the same nutrition. During lactation therefore the growth of the foals is not entirely determined by nutrition. Genetic differences make their appearance—D 1 grows less rapidly than a pure Shire foal although receiving the same nutrition. D 2 grows more rapidly than S 6 (pure Shetland), although both are suckled by dams of the same size. This genetic effect is of course not independent of nutrition. Growth in bulk can only be obtained from the food supply, but apparently genetic differences determine the amounts of the food which will be assimilated and retained for the growth of additional tissues.

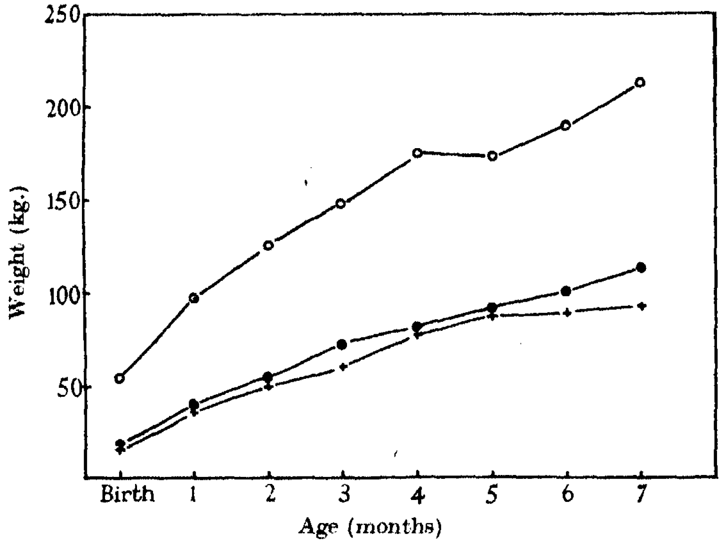


FIG. 1. Early growth of foals. ○ Shetland-Shire cross from Shire dam. ● Shetland-Shire cross from Shetland dam. + Pure Shetland.

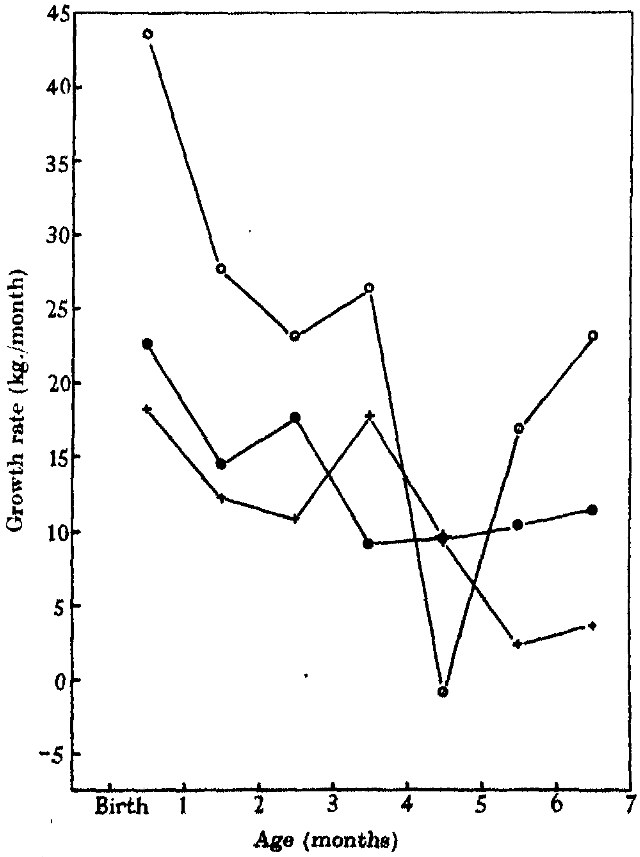


FIG. 2. Early growth rates of foals. ○ Shetland-Shire cross from Shire dam. ● Shetland-Shire cross from Shetland dam. + Pure Shetland.

After weaning the three foals were on a comparable nutrition. D 1, after it had recovered from the temporary set-back due to weaning, continued to grow and make large absolute monthly gains, although, as will be seen from fig. 2, the gains fell off more rapidly than D 2 or S 6. D 2 continued to increase in size relative to S 6. The relationship between the animals is most clearly brought out in fig. 6, which shows the weights of the cross-breds D 1 and D 2 as percentage of the weight of S 6. The curve of D 1 falls and that of D 2 rises rapidly in the early period, but at about 18 months the ratio between all three animals remains remarkably constant. This relationship can also be deduced from a consideration of the percentage increments (fig. 8). In early life the percentage increments of D 2 are greater than S 6 while those of D 1 are smaller. At about 18 months, however, they are approximately equal, and the equality persists in spite

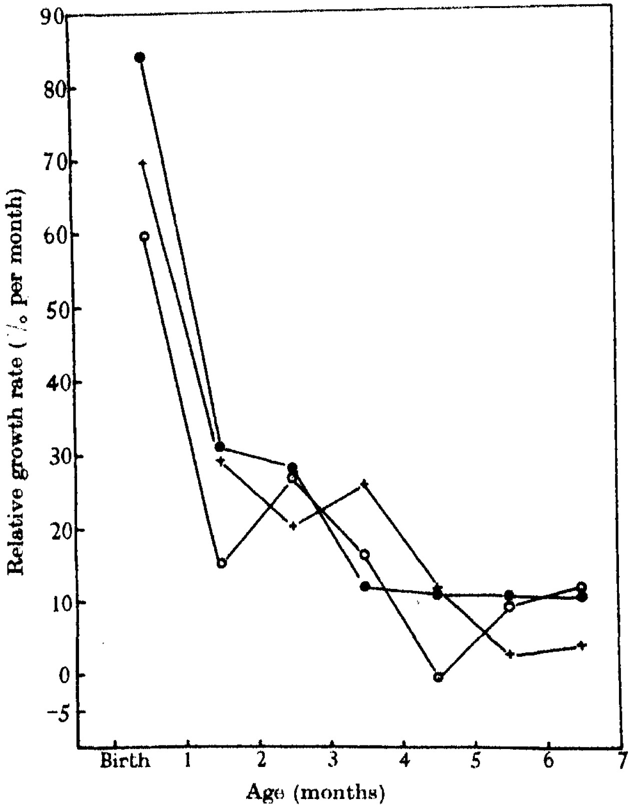


Fig. 3. Early relative growth rates of foals. ○ Shetland-Shire cross from Shire dam. ● Shetland-Shire cross from Shetland dam. + Pure Shetland.

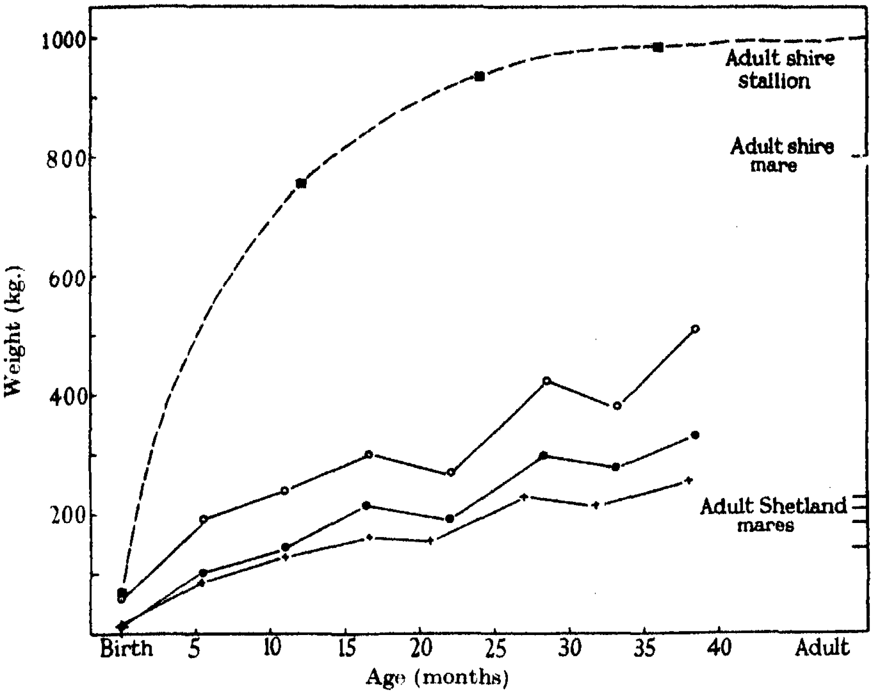


FIG. 4. Growth of foals. ○ Shetland-Shire cross from Shire dam. ● Shetland-Shire cross from Shetland dam. + Pure Shetland. ■ Pure Shire (stallion).

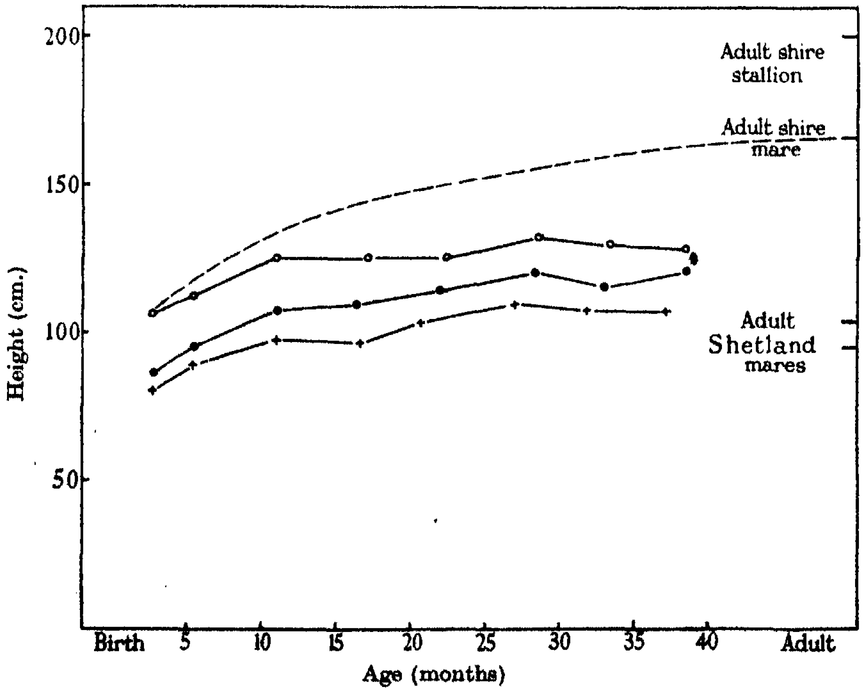


FIG. 5. Growth in height of foals. ○ Shetland-Shire cross from Shire dam. ● Shetland-Shire cross from Shetland dam. + Pure Shetland.

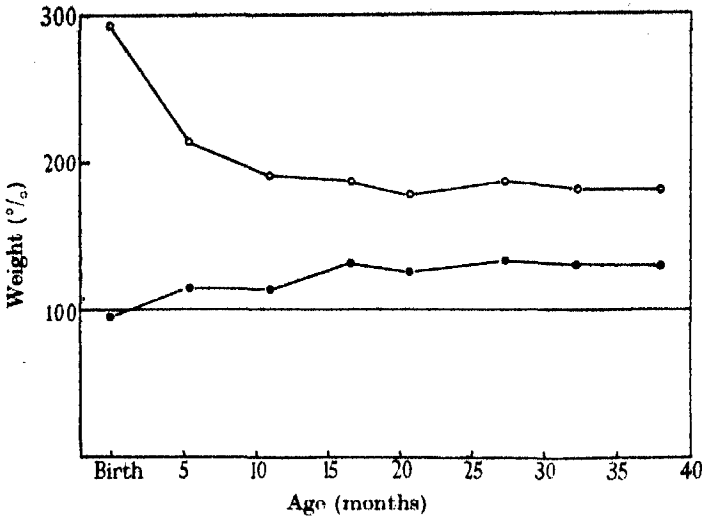


FIG. 6. Weight of cross-breds as per cent of Pure Shetland. ○ Shetland-Shire cross from Shire dam. ● Shetland-Shire cross from Shetland dam.

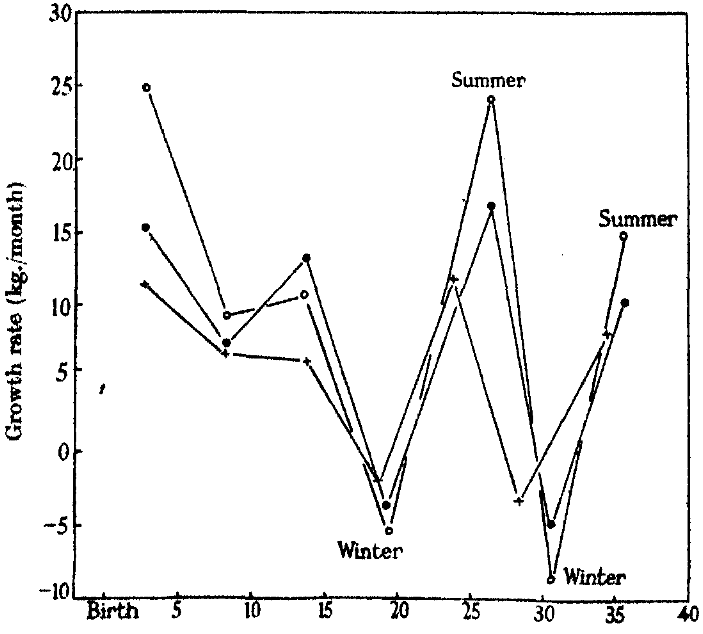
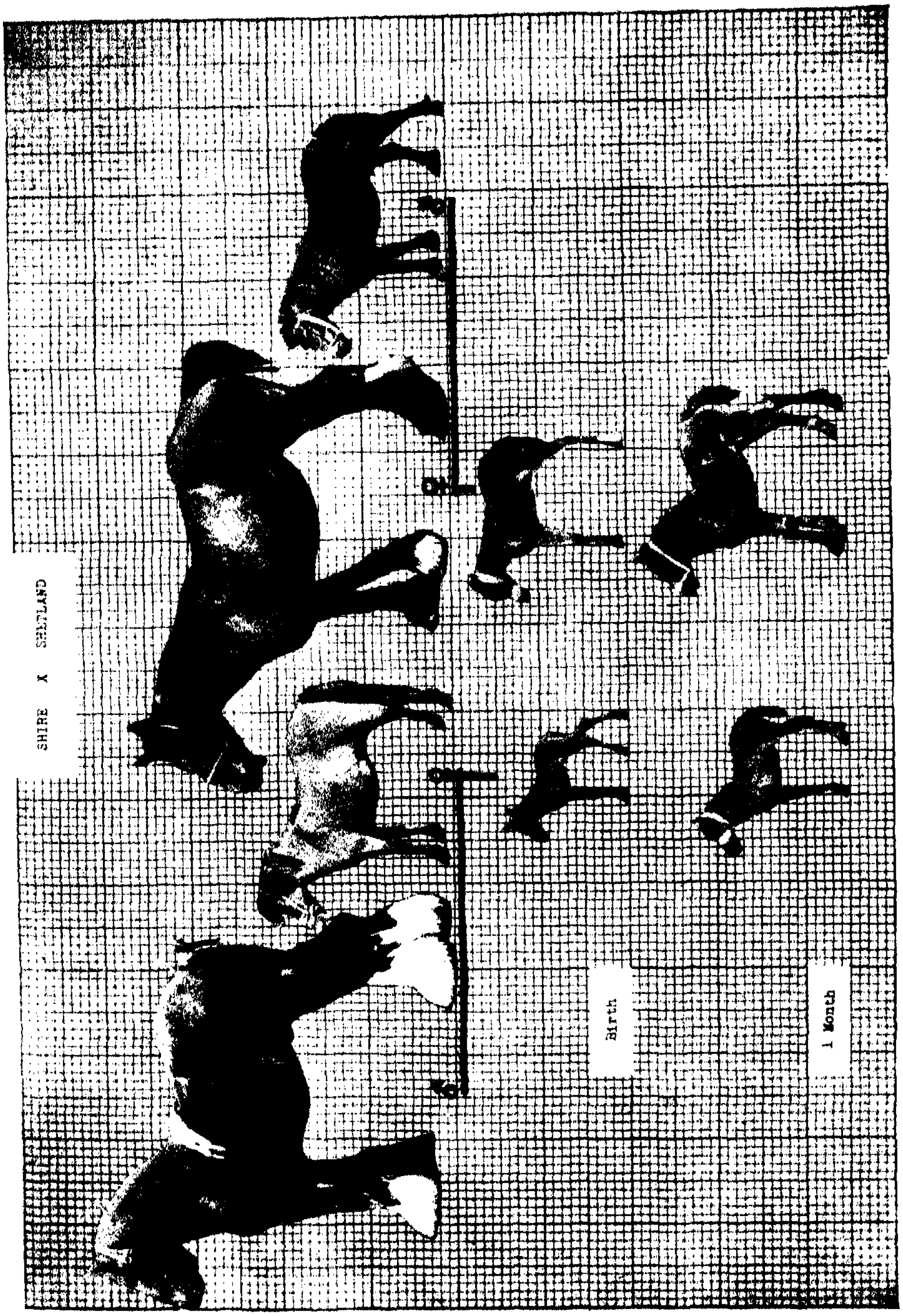
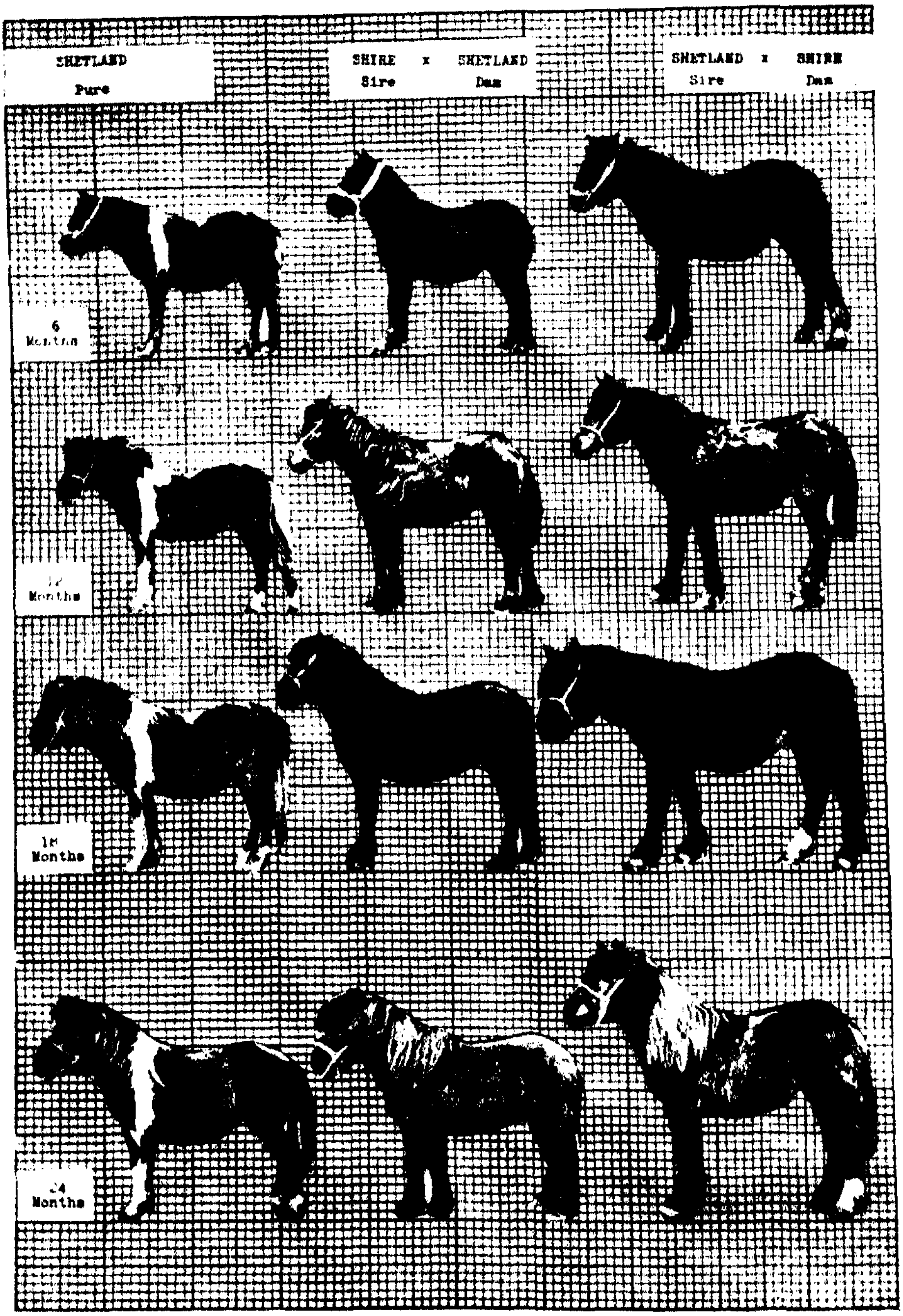


FIG. 7. Growth rates of foals. ○ Shetland-Shire cross from Shire dam. ● Shetland-Shire cross from Shetland dam. + Pure Shetland.



Parents and off-spring of reciprocal Shetland-Shire crosses.



Growth and changes in body conformation of pure Shetland and reciprocal Shetland-Shire crosses.

of the considerable growth that is still being made, and the extreme fluctuation in absolute value of the increments occasioned by seasonal variation in nutrition as shown both by the curves of absolute weight (fig. 4) and monthly gains (fig. 7). What this implies, in other words, is that from about 18 months onwards growth was not influenced by genetical differences and was proportional to the size of the animal already established although subject to considerable fluctuation in nutrition.

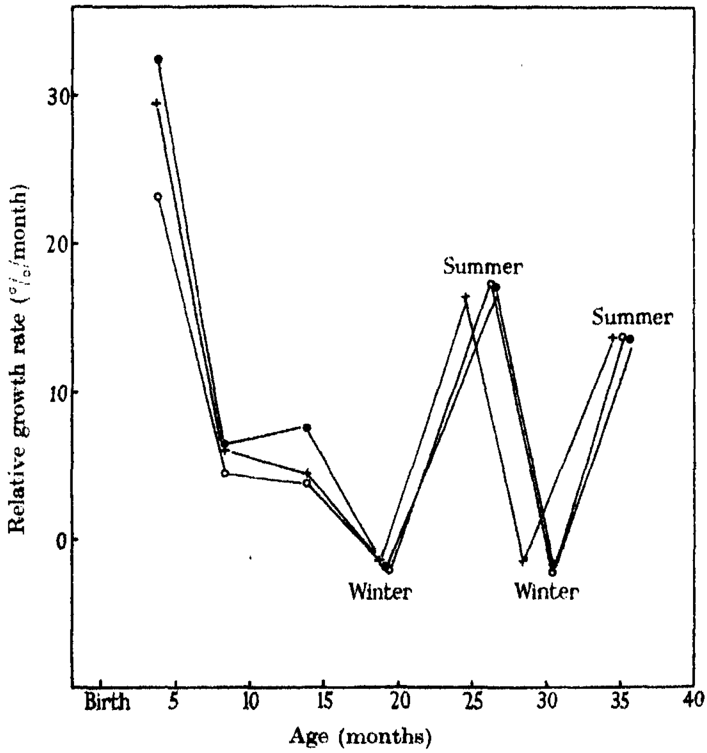


FIG. 8. Relative growth rates of foals. ○ Shetland-Shire cross from Shire dam. ● Shetland-Shire cross from Shetland dam. + Pure Shetland.

The ultimate size which the three foals will attain will not be known for another year or so, but it is obvious from the growth curves that there will be relatively little change in that time and that the ultimate weight is already nearly attained. The pure Shetland, for example, at 3 years old weighs 255 kg., which is above the weight of any of our adult Shetland mares.

In fig. 4 the growth curves of pure Shire are shown for the purpose of comparison. The figures for stallions were given us by Mr A. G. Holland, Secretary of the Shire Horse Society, as being exceptionally good weights. The figure for the mare is that of the mother of D 1. We have not had an opportunity of making more extensive measurements, and the data and the curves should be regarded as only rough approximations. They serve their purpose for making a rough comparison. Now if we compare the weights of the cross-breds with those of the parents we see that while the weight of the cross-bred from the large mother is nearly intermediate, the mean weight of the cross-breds together lies much closer to the Shetland than to the Shire parent. In fact, the difference between the Shetlands and the cross-breds is about 25 % of the difference between the parents. This might be interpreted as indicating dominance of small-size genetic factors over large, but this does not necessarily follow. The bulk of a solid body which increases in size but retains the same relative linear proportions does not increase at the same rate as the linear measurements. For example, the volume of a cylinder is derived from the formula $v = \pi r^2 h$. Hence a cylinder which was exactly half-way in linear dimensions between two other cylinders would be much less than half-way in volume. Now when we compare the cross-breds with their parents in respect to a linear measurement, e.g. height (see figure 8), we find that the discrepancy from intermediacy is much less. The difference in height between the Shetlands and the cross-breds is about 40 % of the difference between the parents. This leaves only 10 % to be accounted for as possibly due to dominance, but a linear measurement may be affected by heterogonic growth (see p. 325), and we are still not in a position to state whether the value we obtain represents at all quantitatively the influence of dominant size genes.

BODY PROPORTIONS

In order to study the proportions of the foals and the changes during postnatal growth a number of measurements were taken at intervals. Linear measurements taken on the surface of the body of a living animal are neither so accurate nor so discriminating of growth differences as measurements by weight. As has been pointed out earlier, the weight of a body roughly cylindrical and maintaining approximately the same linear proportions increases geometrically where a linear measurement would increase arithmetically, but in addition to this there are several sources of error involved in getting the animal to stand still in exactly the same position, in defining exact points of reference on the surface of the body from which measurements are taken and in variation due to thickness of hair, dirt, and so forth. The data are recorded in Table III. Height at withers is the vertical distance from the crest of the withers to the ground with the animal standing four-square. It was taken with a measuring rod with horizontal side arm. Heart girth is the circumference of the trunk, just behind the forelimb. Length of body was measured from muzzle to poll, poll to withers and withers to root of tail. Each measurement was recorded separately, but the sum only is shown in the table. Similarly, length of forelimb was measured from withers to point of shoulder, point of shoulder to elbow, elbow to knee, knee to fetlock and fetlock to ground. Similarly, the hind-limb was measured from middle of rump to stifle, stifle to hock, hock to fetlock, fetlock to ground. Right and left sides of the body were measured separately. The figures in the tables represent the mean of the two sides. In order to determine the fineness of bone, measurements of the circumference of the forecannon, knee, hindcannon and hock were taken on each side of the body and the means recorded in the table. As a measure of comparative fineness all four measurements are added and related to the length of hindcannon.

As a measure of the "stockiness" of the animal the heart girth in relation to height at withers may be taken. The ratio increases in all animals from about 110% at 3 months to about 150% in the adult. In the larger animals it is slightly greater. In thickness of bone there is also a steady increase with age, and again the larger animal has the thicker bone. The cross-breds therefore tend towards the respective proportions of their mothers, but since the size of the animal affects also the proportions, it is not clear whether size or specific genetic influence is responsible. More exact definition is possible in the case of the ratio of length of limbs and length of body. It will be noticed from Table III that the percentage length of limb decreases with age, but in the last few measurements there is a distinct rise. This is, however, not a real increase. As the body fills out and the muscles develop the tape laid on the surface of the limbs from point-to-point becomes more convex and a systematic error is introduced increasing apparently the measurement. For this reason in the analysis which follows only measurements up to 2 years can be relied upon to give an exact picture of growth.

The relationship between the relative growth of the limbs and body is most clearly demonstrated by logarithmic plotting. Fig. 9 shows the length of forelimb plotted against body length and fig. 10 length of hind-limb also against body length. It will be seen that relative growth follows approximately the simple heterogonic formula of Huxley (1932) ($y = bx^K$), indicating that there is a constant ratio between the rate of growth of the

TABLE III. MEASUREMENTS (IN CM.) AND PROPORTIONS OF THE BODY

S 6

Age (calendar months) ...	2·7	5·4	11·0	16·6	20·7	27·0	31·8	37·1
(*a*) Height at withers	80	89	97	96	103	109	107	107
(*b*) Heart girth	85	100	111	124	120	137	135	155
Ratio $b/a \times 100$	106	118	114	129	117	126	126	145
(*c*) Length of body	142	162	177	199	192	197	206	197
(*d*) Length of forelimb	110	121	129	138	139	147	151	153
Ratio $d/c \times 100$	77	75	73	69	71	75	73	78
(*e*) Length of hindlimb	109	117	122	134	132	140	144	143
Ratio $e/c \times 100$	77	72	69	67	69	71	70	73
(*f*) Min. circumference of forecannon	11	13	13	14	14	15	16	15
(*g*) Circumference of knee	18	20	21	22	22	22	24	24
(*h*) Min. circumference of hindcannon	12	13	14	15	15	16	17	17
(*i*) Circumference of hock	22	25	25	27	25	28	29	27
(*j*) Length of hindcannon	28	29	29	30	31	31	31	31
Ratio $(f+g+h+i)/j \times 100$	225	245	252	260	245	262	277	274

D 2

Age (calendar months) ...	2·8	5·5	11·1	16·4	22·0	28·3	33·1	38·4
(*a*) Height at withers	86	95	107	109	114	120	115	120
(*b*) Heart girth	92	107	123	144	134	162	152	175
Ratio $b/a \times 100$	107	113	115	132	118	124	132	146
(*c*) Length of body	152	156	183	197	200	214	205	211
(*d*) Length of forelimb	118	130	142	151	157	161	167	170
Ratio $d/c \times 100$	78	83	78	77	79	75	81	81
(*e*) Length of hindlimb	111	125	133	146	147	155	157	161
Ratio $e/c \times 100$	73	80	73	74	74	72	77	76
(*f*) Min. circumference of forecannon	12	15	14	16	16	16	18	17
(*g*) Circumference of knee	20	22	23	24	24	24	25	26
(*h*) Min. circumference of hindcannon	12	15	15	17	16	18	20	18
(*i*) Circumference of hock	22	25	26	28	25	30	30	30
(*j*) Length of hindcannon	30	32	32	33	31	33	32	33
Ratio $(f+g+h+i)/j$	220	240	244	258	261	267	291	276

D 1

Age (calendar months) ...	2·7	5·5	11·0	16·6	22·2	28·5	33·3	38·6
(*a*) Height at withers	106	112	125	125	125	132	129	128
(*b*) Heart girth	117	134	142	161	141	174	182	188
Ratio $b/a \times 100$	110	120	114	129	113	132	141	147
(*c*) Length of body	178	188	210	226	222	236	239	237
(*d*) Length of forelimb	144	158	169	169	170	180	185	191
Ratio $d/c \times 100$	81	84	81	75	77	76	77	81
(*e*) Length of hindlimb	143	149	162	164	164	172	177	180
Ratio $e/c \times 100$	80	79	77	73	74	73	74	76

TABLE III (*continued*)

(*f*) Min. circumference of forecannon	16	18	19	19	19	20	22	20
(*g*) Circumference of knee	26	27	28	28	28	29	29	30
(*h*) Min. circumference of hindcannon	17	18	19	21	20	22	24	22
(*i*) Circumference of hock	29	33	35	34	31	36	37	36
(*j*) Length of hindcannon	38	38	41	38	41	39	40	39
Ratio $(f+g+h+i)/j \times 100$	232	253	246	269	239	274	280	277

D 3

Age (calendar months) ...	Birth	2·8	5·8	10·8	15·8
(*a*) Height at withers	90	96	115	121	124
(*b*) Heart girth	73	110	129	150	161
Ratio $b/a \times 100$	81	115	112	124	130
(*c*) Length of body	125	166	187	206	216
(*d*) Length of forelimb	120	144	156	170	173
Ratio $d/c \times 100$	96	87	83	83	80
(*e*) Length of hindlimb	116	139	151	161	167
Ratio $e/c \times 100$	93	84	81	78	77
(*f*) Min. circumference of forecannon	12	15	17	18	18
(*g*) Circumference of knee	21	24	26	28	28
(*h*) Min. circumference of hindcannon	12	16	18	20	20
(*i*) Circumference of hock	23	28	32	32	33
(*j*) Length of hindcannon	33	36	37	38	37
Ratio $(f+g+h+i)/j \times 100$	206	230	251	258	268

	S 1	S 3	D 4	D 5	"Daisy"	Sh 1	Sh 2
Age ...	Adult	Adult	Birth	Birth	Adult	Birth	Birth
(*a*) Height of withers	104	95	77	64	166	95	110
(*b*) Heart girth	151	113	55	43	210	83	94
Ratio $b/a \times 100$	145	119	71	67	126	87	85
(*c*) Length of body	202	187	109	102	318	122	143
(*d*) Length of forelimb	145	134	94	82	234	134	138
Ratio $d/c \times 100$	72	72	86	80	74	91	97
(*e*) Length of hindlimb	131	120	96	80	215	133	140
Ratio $e/c \times 100$	65	64	88	78	68	109	98
(*f*) Min. circumference of forecannon	14	12	10	7	29	14	17
(*g*) Circumference of knee	21	19	16	13	41	26	29
(*h*) Min. circumference of hindcannon	14	13	10	8	34	15	17
(*i*) Circumference of hock	25	23	19	16	49	31	36
(*j*) Length of hindcannon	29	29	28	24	48	37	41
Ratio $(f+g+h+i)/j \times 100$	255	231	196	183	319	232	241

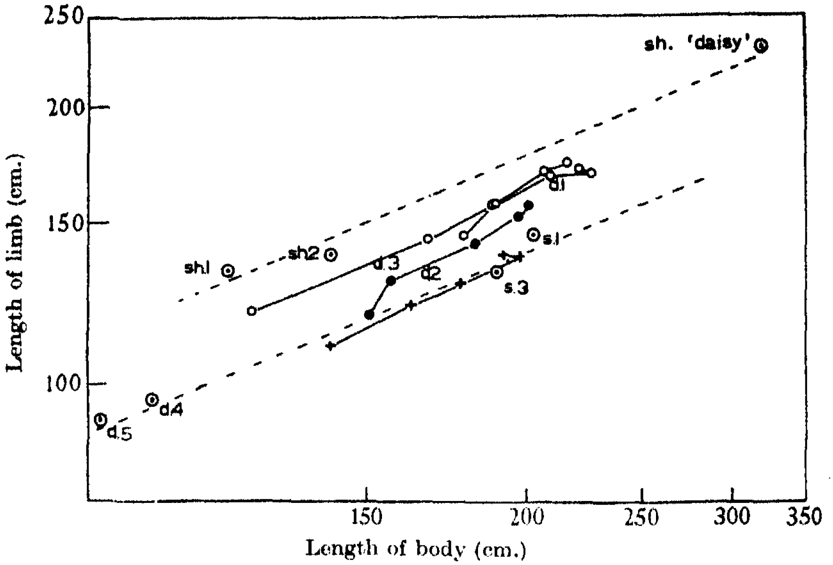


Fig. 9. Relative growth of forelimb to body length, (logarithmic plotting). ○ Shetland-Shire crosses from Shire dams. ● Shetland-Shire crosses from Shetland dams. + Pure Shetland. ⊙ Single point observations.

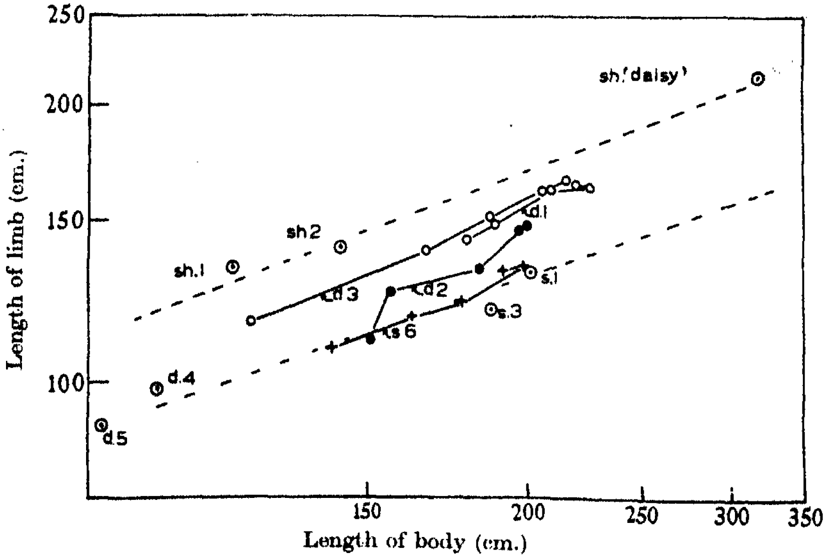


Fig. 10. Relative growth of hind limb to body length (logarithmic plotting). ○ Shetland-Shire crosses from Shire dams. ● Shetland-Shire crosses from Shetland dams. + Pure Shetland. ⊙ Single point observations.

limbs and the rate of growth of the body. *K*, the ratio constant which determines the slope of the curves, is approximately the same in all animals, the possible exception being D 2, the cross-bred from the small mother; this will be discussed later. There is, however, a considerable difference in the fractional constant *b* as denoted by the distance apart of the curves. In words this implies that the pure Shetland is relatively shorter in the limb than the pure Shires and the crosses are intermediate, and that this is true at any body length. For example, at body length 160 the values read from the graph are approximately: Pure Shetland, 121; D 1, 132; D 3, 140; Shire, 155 for the forelimb; and Shetland, 116; D 2, 126; D 3, 135; and Pure Shire, 155 for the hindlimb. With regard to the relative influence of genetic and nutritional factors it is to be noted that the cross-breds occupy an intermediate position between the Shetland and the Shire but are themselves different. D 2 at 3 months and D 4 and D 5 at birth, have a body limb ratio which falls on the pure Shetland curve, but at 6 months D 2 definitely reaches a higher level and follows a line distinctly above that of the pure Shetlands. Up to birth therefore the influence of maternal nutrition predominates, and not until after birth do genetic differences appear to affect the body limb proportions. On the other hand, in D 3 at birth, and in D 1 at 3 months, the proportions are not similar to those of the Shire but distinctly nearer the Shetland type. This shows that the proportions of the body have been affected by genetic differences acting before birth. Subsequently they follow the curve of heterogonic growth established at this early period.

DISCUSSION

In mammals parturition occurs at a time when the foetus reaches a size characteristic of the species and is still able to pass through the genital passage. In most species this size approaches the maximum and parturition is laboured and frequently critical. There is undoubtedly synchronization between the development of the foetus and the termination of pregnancy. The advantage of this synchronization to the animal is obvious and is no doubt an evolutionary adaptation. In the large and small mothers in these experiments the foetus at birth is approximately proportional to the size of the mother, and this proportion is not significantly altered in the reciprocal crossings. Although the offspring differ genetically from their respective maternal parents, as is shown by subsequent growth, at birth the size of the foetus is approximately that to which the mother would normally give birth. The degree of maternal regulation of size in these experiments is very marked. It is, however, not absolutely complete; the cross-breds from the large mother are perhaps not quite so large at birth as normal shires, and the limbs are relatively shorter as seen from figs. 9 and 10. In the small mothers regulation appears to be more complete.

In agricultural practice there is a division of opinion whether the mating of large sires to small dams is liable or not to cause dystokia, although up to the present no critical experiments have been done to test the point. Possibly those cases in which difficulty at birth is experienced are to be attributed not so much to the general size of the offspring as to a disproportionate lack of regulation of some particular part which may give rise to difficulty at parturition, for example the heavy head and shoulders of the Bison cattle-crosses, or the width of skull in certain breeds of sheep and in bull dogs. Difficulty in parturition, although attributed to the size of the foetus, may be due to an abnormal pelvis or to lack of development of the maternal reproductive tract. For example, in the Dexter breed of cattle the "bull dog" monster calf is actually smaller than the normal calf of the same age, but, as it is usually aborted before term, parturition is difficult owing to lack of development of the mother's cervix and vagina.

The mechanism of maternal regulation must remain at present a matter for speculation. There is ample proof in the rabbit (Hammond 1935) that the size of the young is not limited by uterine space, for one horn of the uterus is capable of accommodating double the normal number of young, and at birth the young are not smaller than an equal number of young distributed throughout two horns. In species which are normally monotocous, multiple births are not uncommon, and in these cases the total content of the uterus exceeds that of a single birth, showing that considerable distention is possible. It does not appear probable that uterine surface and placentation are determining factors. In the rabbit experiments quoted above, the placentas were not smaller in the crowded uterus than normal. The size of the placenta is very closely correlated with the size of the foetus, but it is not affected by the space available in the uterus. The foetal placenta is an organ of the foetus and varies with the foetal weight, and is affected by the same changes as the foetus itself.

To look for the causes of maternal regulation, therefore, we must go beyond the spacial and anatomical features of the uterus and placenta. Regulation may take place through limitation in the supply of nutrient material to the foetus. On this hypothesis the growth of the foetus may be determined by the level of nutrient substances available, and this level in turn may be a function of the size of the mother. This explains the well-known fact that in polytocous species the larger the litter the smaller is the individual young, as due to competition for a common food supply. The hypothesis is not upset by the fact that in large litters the total uterine content is greater than in small litters. It is a feature of nutrition that the law of diminishing returns operates so that the larger the litter and the smaller the young the more efficient will be the use made of available nutrients.

Against the hypothesis may be cited the fact that the growth of young *in utero* is not markedly affected by increasing or decreasing the supply of food given to the mother. This relative immunity of the foetus is, however, by no means absolute (see Hogan 1928). Furthermore, if the mother is fed on a low plane of nutrition the foetus will take priority of the available nutrients and deplete the food reserves in the mother's body. On the other hand, if the mother is well fed food reserves will be laid down. The availability of nutrients to the foetus may remain relatively constant whether the mother is gaining or losing weight.

So far we have discussed nutrition in very general terms, but we know that the organism requires a variety of special substances, some of which although small in amount have marked effects upon growth. The vitamins and growth hormones fall into this category. It is possible that regulation of foetal size is brought about through one, or more than one, of these substances. For example, it is possible that growth-promoting hormone of the maternal pituitary may be the regulating factor. This hypothesis is attractive as it at once suggests a mechanism by which the mother's own size and that of the foetus she bears may be co-ordinated.

Another possible explanation of maternal regulation may be cytoplasmic inheritance. It is possible that the ovum receives a quantity of some growth-regulating substance proportional to the size of the mother, and that this determines the size that the foetus will reach at birth. Marshak (1936) found evidence of cytoplasmic inheritance in reciprocal crosses in mice. Cytoplasmic inheritance, however, will not alone explain why after birth the growth rates of the crosses change so that the foals follow a different growth curve to that of the pure-bred foals of the respective maternal parents. This change can, however, be explained on the assumption that maternal regulation acts continuously on the foetus during uterine life and ceases on the termination of pregnancy. It is the uterine environment rather than factors inherent in the foetus which determines regulation.

After birth maternal regulation ceases, perhaps not entirely, since difference in milk supply from the large and small mother may still exert some influence on the growth rate, but after weaning the foals are on the same plane of nutrition. The growth curves tend to converge. The relative increments of the foal from the large mother are less than the relative increments of the foal from the small mother. At about 18 months (see figs. 6 and 8) equilibrium is established and the relative increments remain constant, in spite of considerable fluctuation in absolute growth rates (see fig. 7) due to season variation. It is clear, however, that the differences induced by maternal regulation during pregnancy are not entirely lost, and that the foals are permanently affected, at least as far as the experiment to date carries us. This permanent influence of maternal regulation is not general in all animals (see Green 1931), possibly the long period of gestation, the relatively late stage of development at which the embryo is born and the relatively large difference in nutritional level between the large and small mothers may in this experiment have determined the permanency of the effect.

The marked influence of nutrition on growth which we see illustrated in these experiments is not without theoretical importance. Robertson (1923) and more recently Brody (1928) have made attempts to analyse the integral growth curve in terms of some fundamental intrinsic reaction. This assumes that the organism can be regarded as an isolate in a neutral environment, and that nutrition does not limit growth nor set the pace. This is clearly not the case in this experiment, and further evidence on this point is obtainable from the numerous nutritional experiments the object of which is to improve upon the ration and obtain maximum growth. Until this hypothetical perfect ration is obtained and applied throughout the growth period including pregnancy it cannot be shown that the growth curve is determined by intrinsic reactions and independent of the food supply.

With regard to the genetical implications of the experiment we see that at birth as far as weight is concerned the cross-bred young show little influence of their mixed heritage. Maternal regulation has masked the genetical differences, which, however, appear shortly after the maternal regulation is withdrawn. At about 18 months onwards also, growth is apparently proportional to the size of the animal, and genetical differences do not appear to affect the course of growth. The question of dominance of size factors has already been discussed (see p. 324). In regard to the proportions of the body it is clear that before any comparison of genotypes can be made the influence of size as such must be taken into consideration. Figs. 9 and 10 show that as far as fore and hindlimb lengths are concerned there is a simple heterogenic relationship with body length. The difference of genotype appears however in the difference in the fractional coefficients. To the same body length the Shetland pony is shorter in the limb than the Shire, and the reciprocal crosses are intermediate. The influence of nutrition is not so apparent as in the case of the weight curves. D 2, D 4 and D 5 (foals from the small mothers) perhaps show maternal regulation at birth, and D 2 shows a "genetic" tendency away from the maternal parental type at 6 months. D 3 and D 1 (foals from the large mother) show no definite signs of maternal regulation. The difference between D 2 on the one hand and D 1 and D 3 on the other, is, however, a nutritional effect if our original postulate of genetic similarity (see p. 312) is allowed. The fact that body proportions (bone growth) behaves differently from weight (muscle growth) may possibly be related to the difference in time of maximal development of these two tissues. This experiment well illustrates the interplay of genetic and nutritional factors which are involved in the development of the animal. When offspring are obtained from the cross-bred foals it is hoped that the analysis may be carried further.

ACKNOWLEDGEMENTS

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SUMMARY

1. Reciprocal crosses between the large Shire horse and the small Shetland pony have been made by means of artificial insemination.

2. At birth the foals were approximately proportional in weight to the weights of their mothers and about equal to foals of the pure breeds to which the mothers belonged. The cross-foals from the Shire mare were three times the size of the cross-foals from the Shetland mares. Maternal regulation of foetal growth was very marked and obscured any genetic differences.

3. After weaning, when the foals were under the same nutritive conditions, genetic differences appeared. The foals from the Shire mares grew much less rapidly than pure Shire foals, and the foals from the Shetland mares grew much more rapidly than pure Shetlands. At about 18 months

an equilibrium point was reached at which the relative growth rates of the cross-foals and the pure Shetland remained constant. At 3 years the difference between the reciprocal crosses is still marked and is apparently permanent.

4. Differences in the proportions of the animals, when size differences are eliminated, were not so marked as differences in weight and the influence of nutrition not so obvious.

5. The mechanisms, by which maternal regulation may be brought about, are discussed and three possibilities suggested: (*a*) Maternal regulation of foetal nutrition; (*b*) Maternal hormonal control; (*c*) Cytoplasmic inheritance.

6. The bearing of these results on the theoretical concept of growth is discussed.

7. The experiments illustrate the interplay of nutritional and genetical factors which are involved in development.

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DESCRIPTION OF PLATES

Plate 14. Parents and offspring of reciprocal Shetland-Shire crosses (all to same scale).

Top line	Parent	Shire ♂ × Shetland ♀	Shire ♀ × Shetland ♂
Middle line	Offspring at Birth	Crossbred foal D 2	Crossbred foal D 1
Bottom line	Offspring at 1 month	Ditto	Ditto

The animals were photographed against a squared background, and prints made with the squares enlarged to the same size. Each animal was then cut out from the prints and all mounted on squared paper and rephotographed. Relative sizes and body proportions can be compared.

Plate 15. Growth and changes in body conformation of pure Shetland, and reciprocal Shetland-Shire crosses (all to same scale).

	Pure Shetland (S 6)	Cross-bred from Shetland dam	Cross-bred from Shire dam
Top line 6 months	,,	,,	,,
2nd line 12 months	,,	,,	,,
3rd line 18 months	,,	,,	,,
4th line 24 months	,,	,,	,,

Method of photography and reproduction as in Plate 14.

612.313

The secretion of protein material in the parasympathetic submaxillary saliva

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This paper deals with a study of the secretion of protein material by the submaxillary gland of the cat in response to electrical stimulation of the chorda tympani nerve. In the attempt to make a more generous use of certain advantages offered by physical methods in research on the digestive glands, the submaxillary gland was chosen for the initial investigation because of the relative ease of physiological experimentation. The study is characterized by (1) the use of quantitative spectroscopic methods of analysis which permit an extension in the range of the investigation over that possible with a purely chemical procedure, and (2) the application of mathematical analysis in the interpretation of the results.

The work of previous investigators (Heidenhain 1868, 1878, 1883; Werther 1886; Langley and Fletcher 1890; Gregerson and Ingalls 1931) has shown that with increasing intensity of the stimulus the parasympathetic submaxillary saliva becomes more concentrated in (1) inorganic salts which are presumably derived from the tissue fluids and ultimately from the blood, and (2) organic material, provided that the stores in the gland are not too much depleted by previous stimulation. The concentration changes in the former group of substances are presumably connected with phenomena occurring at the glandular membranes; those in the latter group obviously cannot be accounted for by a simple washing out of stored material from the gland, but must result from the operation of a more complicated secretion mechanism. In the attempt to gain a better understanding of the fundamental nature of the secretion processes, we have determined spectroscopically the relative concentrations of protein material, and the Na and K concentrations in samples of saliva obtained in response to various intensities of stimulation of the chorda tympani nerve. Many of the samples were also analysed chemically for protein and non-protein nitrogen, chlorides, and acid-combining power. These substances, along with water, are the main constituents of the submaxillary saliva as obtained in our experiments. A long series of *relatively small* samples of secretion was taken from each animal; this feature of the experimental technique is considered to be important, viz. under such conditions one may best determine the changes in composition resulting from the variation of one factor only—the intensity of the stimulus—since changes in other factors which are not directly controlled are small from sample to sample.

It has been possible to develop a mathematical expression which closely describes the relative concentrations of protein material in series of samples of saliva obtained in response to widely varied intensities of the stimulus. The considerations involved indicate that there is an interrelation between the mechanism responsible for the secretion of protein, that responsible for the secretion of water, and that controlling the membrane phenomena. A reasonable inference is that the operation of all three mechanisms depends on the rate at which some activating substance is liberated within the glandular cells by the stimulation. The mathematical expressions developed permit the interpretation that during stimulation part of the granule material is transformed in an irreversible chemical reaction to a form which is readily carried out of the cells by the flow of water.

1. EXPERIMENTAL PROCEDURE

(*a*) *Physiological technique.* Cats under nembutal anaesthesia were used in all experiments. They were tracheotomized and the submaxillary ducts were cannulated. The chorda tympani and cervical sympathetic nerves were sectioned and taken on ligatures. Rhythmic stimulation of the chorda tympani nerve (stimulation period, 1 sec.; rest period, 4 sec.) was carried out with a Palmer coil controlled by a metronome. Such stimuli were applied for 5–10 min. periods followed by 5 min. intervals of rest. The periods were kept constant for all samples taken for analysis in an experiment. The saliva was collected through a pyrex cannula into graduated pyrex test-tubes.

The nerve was placed on the electrode at the point where it left the lingual nerve, and once adjusted was not moved until the end of the experiment. After each period of stimulation it was washed with 3-5 c.c. of physiological saline, in order to remove any possible metabolic products of nerve activity and of polarization. At all other times cotton moistened with saline solution was wrapped about it in such a way as not to touch the point of contact of nerve and electrode. The whole was kept warm by an electric lamp placed above it. When this procedure was not adopted the observed volume of secretion obtained with constant coil conditions decreased markedly as the experiment progressed.

(*b*) *Analysis of the samples.* The absorption spectrum of the saliva consists of the so-called protein band near $\lambda 2800$, and end-absorption near $\lambda 2500$. Change in the intensity of the stimulus merely alters the strength of the absorption. The intensity ratio of the incident to the transmitted light in the $\lambda 2800$ region was determined for each sample by a simple photographic method employing rotating step-sectors and requiring less than 0·5 c.c. of secretion. The measurements were made immediately on completion of the physiological experiment in order to avoid the changes in absorption which are characteristic of most protein solutions on standing for an appreciable period. Preliminary tests showed that this procedure was adequate. Once the intensity ratio of incident to transmitted light is determined for the $\lambda 2800$ region, a value for the product of the extinction coefficient (ϵ) and the concentration (c) may be calculated from the ordinary absorption law. Since chemical analyses on large quantities of saliva indicate that over 90% of the protein composition is mucin, and since other components do not absorb strongly in the $\lambda 2800$ region, the measured absorption may be taken to be due to the mucin; the ϵc values calculated from the absorption law therefore correspond to the relative concentrations of mucin, providing Beer's law holds for the secretion. That it does is indicated by the fact that the extinction coefficient as calculated per unit weight of protein nitrogen (determined chemically) is found to be essentially constant over a wide range. The average deviation from the mean for eleven samples in which ϵc varied from 0·30 to 1·7 per cm. path, was less than 7 %.

Na and K were determined by a spectroscopic method (a description of the method is in process of publication: McRae, Langstroth, and Foster 1938). A complete analysis consisted of a double determination for each element, and required only 0·25 c.c. of saliva. The estimated precision for a single determination is better than 10 %; in forty pairs of determinations on saliva the average deviation of the observed values from the mean was less than 5 %.

Nitrogen determinations were made by the micro-Kjeldhal method (Pregl 1930) on the precipitate obtained by treating the saliva with acetone containing some acetic acid; they were also made on the protein-free filtrate. Acid-combining power and chlorides were determined respectively by Pickerell's (1914) method, and by Wilson and Ball's (1928) modification of Van Slyke's method.

2. Experimental results

The results of a typical experiment are illustrated in part in Table I. In addition to the figures given, protein and non-protein nitrogen were determined in eight samples, chlorides in five samples, and acid-combining power in nine samples. The changes in the protein nitrogen concentration closely parallel the changes in ϵc as previously stated, while the sum of the chloride concentration and the acid-combining power is approximately equal to the (Na + K) concentration, in milliequivalents per litre. The individual variations in the concentrations of these substances will be referred to in another article. The results of other experiments are given in a more abbreviated form in the following section.

In the experiment of Table I rhythmic stimulation was applied for 6 min. periods followed by 5 min. intervals of rest. With weak stimulation (i.e. large-coil distance) several samples so obtained were mixed together in order to have sufficient saliva for convenient analysis; this has been indicated under the heading "No. of samples". On each change in the strength of the stimulus a small sample was taken to clear the ducts of secretion due to the previous stimulation. These samples, which ranged from 0·2 to 0·5 c.c. in volume, were discarded. The Na and K concentrations are given in milliequivalents per litre. The ϵc values are purely relative. The order in which the samples are given is the order in which they were obtained. This is important in view of the progressive exhaustion of stored material which takes place during the experiment.

TABLE I. EXPERIMENT 23A. LEFT SUBMAXILLARY GLAND

Coil distance cm.	No. of samples	Rate of secretion c.c./6 min.	ϵc	Na	K
20	2	0·45	0·68	7·4	11·7
16	1	1·4	1·10	24·4	7·9
22	3	0·25	0·59	8·7	15·8
13	1	2·0	1·26	39·1	8·4
24	6	0·20	0·54	10·4	15·8
10	1	3·0	1·64	60·9	9·9
20	1	1·3	0·72	23·5	8·7
8	1	2·6	1·24	52·1	9·9
10*	1	2·6	0·76	45·2	10·7
16	1	1·5	0·54	27·4	11·0
20	1	0·85	(0·29)	14·8	10·4
10*	1	2·6	0·86	50·4	10·4

* No sample taken to clear the previous secretion from the ducts.

Fig. 1 illustrates some of the features of the secretion of Na, K, and water, which have a bearing on the interpretation of the data for the relative concentrations of protein material, as will appear later. With rates of flow of water greater than about 1·2 c.c. per 6 min., the Na concentration, and the (Na + K) concentration, are linearly related to the rate of secretion of water, while the K concentration is essentially independent of it. Furthermore, the (Na + K) concentration is directly proportional to the rate of flow, i.e. the extrapolated line passes through the origin. With lower rates of flow, however, the Na and K concentrations are greater than expected on the basis of these relations. It may be added that an exactly similar departure from the expected values is observed for the chlorides and acid-combining power. The unexpectedly high *concentrations* of *all* these dissolved substances at low rates of flow are not unreasonably interpreted as due to the operation of some disturbing factor—such as a reabsorption of water—which results in a diminution in the water output, but does not appreciably affect the *amounts* of the dissolved substances secreted. This interpretation is supported by the fact that unexpectedly high concentrations of K are observed at low rates of flow, although at higher rates the K concentration is practically independent of the rate. This interpretation is consistent with the considerations of the following section.

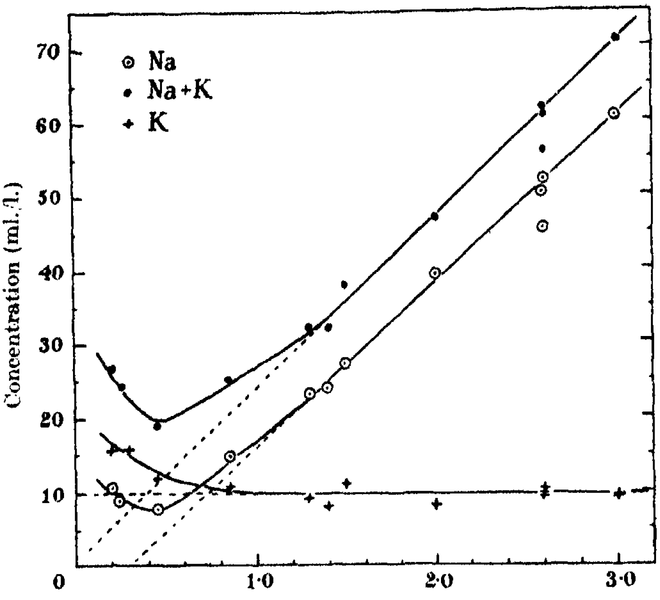

FIG. 1

3. MATHEMATICAL TREATMENT OF THE SECRETION OF PROTEIN MATERIAL

There is little doubt that the protein material secreted in the submaxillary saliva has its origin within the secretory cells, since the membranes do not normally permit the passage of large molecules from the tissue fluids into the secretion in appreciable amounts. Furthermore, the concentration of protein material in the saliva decreases with prolonged stimulation and may reach very low values. This decrease is accompanied by a gradual disappearance of the granules from the cells, and restoration of the granules during rest is known to take place very slowly as compared to the rate at which the stores are depleted in the present experiments. Consequently, in dealing with the data of these experiments, the rate of formation of granule material may be taken to be negligibly small, and need not be considered.

It is an observed fact that an increase in the intensity of the applied stimulus results in an increase in the rate of secretion of (1) water, (2) inorganic salts, and (3) protein material, i.e. the output of each of these three constituents in a given time under a definite set of conditions should be expressible as some function of the intensity of the stimulus. These functions cannot be determined from the data because the intensity of the stimulus at the gland is not known. It should be possible, however, to express the changes in the output of one constituent brought about by varying the intensity of the stimulus, in terms of the changes which take place in the output of either of the other constituents. From such perfectly general considerations therefore, one may hope to be able to express the rate of secretion of protein material in terms of the rate of secretion of inorganic salts, or of water. It will also depend, of course, on the amount of the parent substance (i.e. the granule material) present in the gland at the time under consideration.

An expression for the rate of secretion of protein material which has been found to lead to a quantitative description of the protein concentrations in the samples of critical experiments, is

$$dN/dt = -kNS, \tag{1}$$

where N represents the amount of granule material present in the gland at the time, S denotes the rate of secretion of (Na + K) in milliequivalents per minute, and k is a constant. Since no other metal appears in the saliva in concentrations comparable to (Na + K), S corresponds very nearly to the total amount of metals (and also of inorganic salts) secreted per unit time.

Integration of (1) gives

$$N = N'e^{-kSt}$$

as the amount of granule material N, left in a gland which originally contained N', but which has secreted under constant stimulus for a time t. The amount of protein material secreted is

$$\Delta N = N' - N = N'(1 - e^{-kSt}).$$

In general, if N_0 be the amount of granule material present in the gland at the beginning of an experiment in which a series of samples of secretion are taken under different intensities of the stimulus, then the amount left in the gland at the beginning of the ith sample of the series is

$$N_{i-1} = N_0 \exp\left[\sum_{r=1}^{r=i-1} kS_r t_r\right]$$

where S_r denotes the rate of secretion of (Na + K) in the rth sample collected in a time t_r. The amount secreted in the ith sample is

$$\Delta N_i = N_0 \exp\left[\sum_{1}^{i-1} kS_r t_r\right](1 - e^{-kS_i t_i}). \tag{2}$$

Under the conditions of our experiments, the exhaustion per sample was sufficiently small to permit the neglect of squares and higher powers in the series expansion of simple exponentials of (2). Hence

$$\Delta N_i = N_0\, kS_i t_i \exp\left[\sum_1^{i-1} kS_r t_r\right]. \tag{3}$$

Since the concentration of protein material is obtained by dividing ΔN_i by the volume of the sample V_i, the relative concentrations of protein material in the ith and nth samples, reduce to

$$C_i/C_n = \frac{S_i t_i}{V_i}\frac{V_n}{S_n t_n}\exp\left[\sum_n^{i-1} kS_r t_r\right]; \quad n < i, \tag{4}$$

which may be written

$$C_i/C_n = D_i/D_n \exp\left[\sum_n^{i-1} kB_r\right], \tag{5}$$

where D_i denotes the concentration of (Na + K) in the ith sample in milliequivalents per litre, and B_r represents the total amount of (Na + K) secreted in the rth sample in milliequivalents.

According to equation (5) the concentration of protein material in any sample depends not only on the experimental conditions under which the sample was obtained, but also on the experimental history of the gland as expressed by the exponential or "exhaustion" factor. To evaluate ΣkB_r one must have appropriate data for all samples taken from the gland between the first and last under consideration. Obviously equation (5) is applicable only when the series of samples is taken without interruption, or when the conditions are such that the restoration of granule material during periods of rest is negligible. In making a comparison with experiment, the value of the single unknown constant k is determined from the observed protein concentrations for two samples, and this value is used to calculate the protein concentrations for all the samples of the series. For the various animals of our experiments, k ranged from $2{\cdot}8 \times 10^{-4}$ to 33×10^{-4}, with an average value of $10{\cdot}5 \times 10^{-4}$. For most animals it lay near the average value.

A comparison of calculated and observed protein concentrations is given in Table II for several typical experiments. In all the data, which include the samples for ten experiments, the average deviation of the calculated from the observed values was less than 10 %. No comparisons were discarded in arriving at this figure.

On the general grounds stated at the beginning of this section, one might expect to be able to develop an alternative treatment of the secretion of protein material by expressing the rate of secretion of protein material in terms of the rate of secretion of water, rather than of the dissolved substances. It is found that the equation,

$$dN/dt = -k'NR^2, \tag{6}$$

where R denotes the rate of secretion of water and k' is a constant, leads to an expression (analogous to equation (5)) which is in equally good agree-

TABLE II. COMPARISON OF CALCULATED AND OBSERVED RELATIVE CONCENTRATIONS OF PROTEIN MATERIAL

Exp.	Sample	Secretion rate c.c./10 min.	(Na + K) millieq./l.	Protein conc. (ϵc)	
				Calc.	Obs.
23A	1	0·75	19·1	0·60	0·68
	2	2·3	32·3	1·0	1·1
	3	0·42	24·5	0·72	0·59
	4	3·3	47·5	1·3	1·3
	5	0·33	26·2	0·66	0·54
	6	5·0	70·8	1·7	1·6
	7	2·2	32·2	0·60	0·72
	8	4·3	62·0	1·0	1·2
	9	4·3	55·9	0·78	0·76
	10	2·5	38·4	0·48	0·54
	11	1·4	25·2	0·28	0·29
	12	4·3	60·8	0·68	0·86
9A	1	1·3	23·9	0·88	0·88
	2	1·3	21·0	0·76	0·68
	5	5·5	45·7	1·6	1·7
	16	1·3	18·2	0·49	0·51
	17	1·4	17·7	0·46	0·46
7A	1	5·2	53·6	2·4	2·5
	2	4·0	45·1	1·5	1·6
	3	3·5	39·7	1·1	0·8
	4	3·0	35·4	0·8	0·8
	5	2·5	28·7	0·6	0·7
	6	2·7	38·2	0·7	0·6
	7	2·0	27·2	0·5	0·5
	8	3·5	44·5	0·7	0·7
	9	3·4	36·0	0·5	0·3
5A*	3	7·0	73·4	2·4	2·2
	6	6·3	56·4	1·5	1·5
	9	4·0	42·2	1·1	1·5
	15	6·5	62·7	1·3	1·3
	18	7·3	63·4	1·0	1·0

* The samples of Exp. 5A were obtained in response to constant, rather than rhythmic, stimulation. The stimulus was applied for 2 min. periods at intervals of 15 min.

ment with observation except for samples with a rate of flow of less than about 1·2 c.c./6 min.; for these samples the expression fails badly. This circumstance is to be expected if the interpretation previously given to account for the unexpectedly high concentrations of dissolved substances at low rates of flow is correct (see fig. 1), i.e. if the output of water is affected at low rates of flow by some disturbing factor such as reabsorption, the *observed* rate of flow of water ceases to be a measure of the glandular activity in the same sense as at higher rates of flow, and obviously must give erroneous results when substituted in an expression which assumes that it is.

4. DISCUSSION

It has been shown that the relative concentrations of protein material in samples of submaxillary saliva obtained in response to chorda tympani stimulation are quantitatively described by equation (5). Hence the rate of secretion of protein material is given in terms of the rate of secretion of (Na + K) by equation (1). Furthermore, it has been found that, for samples having a rate of flow greater than about 1·2 c.c./6 min., the rate of secretion of protein material may also be expressed in terms of the rate of secretion of water by equation (6). The fact that a quantitative relationship exists between the rate of secretion of protein material, of water, and of metals (or inorganic salts), is not surprising in view of the general considerations stated at the beginning of the preceding section. It may be interpreted on the basis that the mechanism responsible for the secretion of protein material, that responsible for the secretion of water, and that controlling membrane permeability, operate at rates depending in perfectly definite ways on the amount of some activating substance liberated within the gland by the stimulation.

As suggested in the introduction, the observed increase in the protein concentration of saliva with increase in the intensity of the stimulus cannot be explained on the basis of a simple washing out of stored material by the flow of water, but must result from the operation of some more complex secretion mechanism. Equation (1), or equation (6), with S or R^2 replaced by the appropriate function of the amount of activating substance present, is similar in form to the velocity equation for an irreversible chemical reaction. This fact suggests that the mechanism responsible for the secretion of protein material consists of a chemical reaction or chain of reactions, resulting in the transformation of granule material to a form readily carried out of the cells by the flow of water. On this view, the rate at which protein material is discharged from the gland is determined by the rate at which granule material is transformed, and so by the amounts of activating substance and granule material present in the gland. It may be stated here that this mechanism is of the same nature as that in operation in the pancreas, as indicated by the results of studies to be published shortly (Langstroth, McRae, and Komarov 1938). The data for the pancreas furnish a relatively severe test of this type of interpretation, since synthesis of granule material in critical experiments cannot be neglected, and since the relative amounts of the activating substance (secretin) present in the gland are known.

The observed increase in the rate of secretion of inorganic substances with increasing intensity of the stimulus is attributed to an increase in the effective permeability of the glandular membranes which is naturally a function of the degree of activity of the gland. The rates of secretion of protein material, and of water, are also functions of the degree of activity of the gland. Since the rates of secretion of water, protein material, and (Na + K), are related in a perfectly definite way as shown in the preceding section, changes in the rate of secretion of (Na + K) (or of total metals) may be considered to provide a measure of the changes in the intrinsic character of the membranes. Changes in the rate of secretion of any one substance, however, do not, e.g. substitution of the rate of secretion of Na for S in equation (1) leads to a marked disagreement between calculated and observed protein concentrations. This is understandable, since the rate of transfusion of any substance through the membrane must depend not only on the intrinsic character of the membrane but also on the concentrations of other substances on either side and on their rates of transfusion. For example, a preponderance of net ionic charge on one side must result in the production of a potential drop across the membrane which facilitates the passage of ions of one sign while retarding those of the other; moreover, such forces must have a greater effect on the passage of ions which encounter little resistance than on that of similarly charged ions which pass with difficulty. On such considerations the best available measure of changes in the character of the membranes are the changes in the rate of passage of, say, anions, which in our experiments are closely represented by the changes in the rate of secretion of total metals. It is significant that S must be expressed in milliequivalents per litre to give agreement between calculated and observed protein concentrations, i.e. either the total number of positive ionic charges, or the total number of particles of Na and K, passing the membranes per unit time is an important factor in the phenomenon.

The relation, S is proportional to R^2, may be obtained from equations (1) and (6) for the rate of secretion of protein material by elimination of $(1/N \,.\, dN/dt)$. Owing to the limitations of (6) this relation may be expected to hold only for samples having a rate of flow greater than about 1·2 c.c./6 min. Data such as that of fig. 1 confirm this relation, i.e. for the higher rates of flow, the concentration of (Na + K) is observed to be directly proportional to the rate of secretion of water.

It should be pointed out that in the considerations of the preceding pages the number of actively secreting cells in the gland was presumed to remain constant during any experiment. Furthermore, it was assumed that a change in the intensity of the stimulus affected equally all the actively secreting cells. Both these assumptions appear to be essentially correct in view of the close agreement between calculated and observed protein concentrations.

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SUMMARY

A quantitative study has been made of the variations which occur in the composition of the submaxillary saliva of the cat when the intensity of chorda tympani stimulation is altered. Spectroscopic methods of analysis, both from emission and absorption spectra, aided considerably in obtaining relatively complete data.

A mathematical treatment of the secretion of protein material has been developed. It leads to the following expression for the relative concentrations of protein material in two samples (n, i) taken from a series in which the intensity of the stimulus is varied,

$$C_i/C_n = D_i/D_n \exp\left[\sum_{n}^{i-1} kB_r\right].$$

k is a constant, D_i denotes the concentration of (Na + K) in the ith sample in milliequivalents per litre, and B_r represents the amount of (Na + K) secreted in the rth sample in milliequivalents. The expression is in remarkably good agreement with the experimental results.

The mathematical treatment permits the following interpretations to be made: (a) the mechanism responsible for the secretion of protein material, that responsible for the secretion of water, and that controlling membrane

permeability, operate at rates dependent on the rate at which some activating substance is liberated within the gland by the stimulation, and (*b*) the mechanism responsible for the secretion of protein material consists of a chemical reaction which transforms granule material to a form readily carried out of the cells by the flow of water.

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612.63:599.32 *Cricetus*

Relation between the uterus and the ovaries in the pregnant hamster

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[Plates 16–22]

1. Introduction

In spite of many experimental investigations on numerous species, it still seems impossible to decide for how long the ovaries or the corpora lutea are necessary for maintaining pregnancy in mammals. As this problem has been thoroughly reviewed elsewhere (Asdell 1928; Parkes 1928; Klein 1934; Kehl 1934; Haterius 1935; Gros 1936), it is unnecessary to give a detailed survey of it here. Apparently mammals can be divided into two groups. In the first group, to which belong the rabbit, rat, mouse, and cow, ovariectomy or destruction of the corpora lutea always results in the termination of pregnancy and abortion at any stage of gestation. In the second group, on the contrary, it is possible to remove the corpora lutea or the whole ovaries a certain time after mating, without interfering with the normal progress of pregnancy and parturition; to this group belong the guinea-pig, cat, mare, and man. It follows that conclusions from experiments carried out on one species do not necessarily apply to another. It may be of interest therefore to describe results obtained on a species which has not hitherto been investigated; in the present paper we shall report experiments carried out on pregnancy in the Syrian golden hamster.

2. Material and technique

The golden hamster, *Cricetus* (*Mesocricetus*) *auratus* Waterhouse, is a native of Syria, introduced into England in 1932; notes on its breeding and growth have been published by Bruce and Hindle (1934), who successfully bred the species. The animals used for the present experiments came from the stock kept at the National Institute for Medical Research, London, where most of our experiments were carried out. We have reared a colony of these hamsters at the Institut d'Histologie, Strasbourg, where the experimental series was completed.

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The pregnancy of the golden hamster, lasting 16 days, seems to be the shortest of the laboratory rodents. This is a disadvantage in some respects, since pregnant females can be operated on only during a short period. Pregnancy can be detected with certainty by palpation from the eighth day onward. The very rapid growth of the embryos between the tenth and fifteenth day, moreover, makes a precise determination of the first day of pregnancy possible by measuring the uterine swellings or the embryos, in cases in which the vaginal plug is not found.

The animals were opened under avertin anaesthesia (1 c.c. of a 2·5% solution per 100 g. body weight intraperitoneally), which proved to be without any adverse effect on pregnancy, and to provide a regular and deep narcosis for about 1 hr.

The substances used for hormone therapy were oestrone, synthetic crystalline progesterone (proluton), kindly supplied by Schering-Kahlbaum A.G., and in a few cases progestin B.D.H. The physiological potency of the progesterone solutions was tested by the McPhail (1934) method on the endometrium of the immature rabbit.

The usual histological routine was followed: fixation in Bouin's fluid, and staining with haematoxylin and eosin. All vaginal sections were, moreover, stained with mucicarmine and methanyl yellow, this method being especially useful to ascertain small amounts of mucus or its absence in the epithelial cells.

3. REPRODUCTIVE CYCLE OF THE NORMAL GOLDEN HAMSTER

The reproductive cycle of the golden hamster has been described by Deanesly (1938); here we shall give only a short summary of the main features of the cycle and mention some special facts in connexion with our experiments.

The normal cycle of the unmated female lasts 4 days. As in the mouse and the rat, the corpora lutea are probably functionless, since in the absence of mating the characteristic change does not appear in the reproductive tract during the luteal phase. It is not possible to check the phases of the ovarian cycle by means of vaginal smear; the lower part of the vagina is always cornified and does not show cyclic changes. Masses of cornified cells are shed off constantly and are pushed out in clots at irregular intervals, often masking the oestrous mucous cell discharge from the upper part of the vagina.

After mating there is a solid bicornuate vaginal plug which may fall out rapidly and sometimes is not easy to distinguish from the cornified cell clot of the lower vagina. For this reason it is occasionally difficult to date a pregnancy or pseudopregnancy exactly. After sterile mating the dioestrous interval lasts about 10 days. The corpus luteum persists for a longer time than that found during the dioestrous cycle, but after the seventh day the luteal cells show definite signs of regression. The corpora lutea of pseudopregnancy are functional: a typical change occurs in the upper part of the vagina which will be described below.

The number in the litters is extremely variable. It ranges from one to thirteen foetuses. The corpora lutea of pregnancy are larger than those of pseudopregnancy but they degenerate rapidly after parturition. No postpartum ovulation or oestrus takes place.

The characteristic histological changes of the vagina which have been used as a convenient morphological test in several groups of experiments will now be described. These changes are restricted to the middle and the upper parts of the vagina, which undergo striking epithelial transformations different in many respects from those described for other species of rodents.

During oestrus the epithelium shows a special structure which has been termed "foliaceous" (fig. 1, Plate 16). It is constituted by two to seven layers of squamous stratified and slightly cornified epithelium. Above them are several layers of loose epithelial cells packed together in tufts. These are irregular epithelial cells in which special stains reveal mucous droplets. After ovulation, the superficial layers are rapidly shed into the lumen, the basal layers of stratified epithelium thicken and numerous polymorphs migrate through the epithelium into the lumen (metaoestrous). The epithelium grows thicker, the cells become cubical or even elongated towards the lumen and slightly columnar, small cysts are formed with mucous droplets, and then the cells loosen, become irregular and the foliaceous appearance is again seen.

After mating the vaginal epithelium undergoes a typical transformation (figs. 2, 3, Plate 16) and it is then constituted by one layer of tall cylindrical cells, completely filled with mucus, with basal nuclei. Beneath this row of mucified cells there is a flattened basal layer. This mucification appears both during pseudopregnancy and pregnancy, and lasts till the next oestrus or parturition. We have previously published an experimental study on the causation of vaginal mucification in the hamster (Klein 1937). We have been able to show that full mucification, as it appears after mating, is caused by the simultaneous action of progesterone and oestrone.

The ovaries are very much like those of the mouse and the rat (fig. 4, Plate 16). Unlike the latter, however, the corpora lutea disappear very quickly after ceasing to function, and only one set is observed at a time.

Finally, it may be added that the mammary gland shows very active epithelial transformations under experimental conditions and has also afforded an interesting morphological test in our experiments. We shall describe the cytological changes of the mammary gland very briefly, as we expect to publish them later with other cytological observations.

4. NATURE OF EXPERIMENTS

First, we wished to discover whether the ovaries are necessary for the progress of gestation between the ninth and the sixteenth days of pregnancy. When it was shown that ovariectomy caused abortion, control experiments were performed in order to exclude operative shock. Then we tried functional replacement of the ovaries in the ovariectomized pregnant female by injections of ovarian hormones. These three groups of experiments establish the necessity of the ovaries and especially of the corpora lutea for the maintenance of pregnancy.

A new problem then arose: why does the corpus luteum of pregnancy last 16 days, whilst outside pregnancy it is functional for a much shorter period? This problem concerning the connexion existing between the pregnant uterus and the ovaries was examined by means of two kinds of operations: removal of the whole pregnant uterus and removal of the embryos only.

These various experiments provide a fairly satisfactory account of some endocrine conditions governing pregnancy in the hamster.

5. THE ROLE OF THE OVARIES IN MAINTAINING PREGNANCY

(*a*) *Effects of ovariectomy on pregnancy*

Eight pregnant hamsters were ovariectomized between the eleventh and thirteenth day of pregnancy. In all cases the complete removal of the ovaries resulted in immediate termination of pregnancy. Therefore, the ovaries are absolutely necessary during the whole span of gestation. In this respect the golden hamster agrees with the rabbit, the mouse, and the rat.

Autopsy after abortion did not always show the same condition. The termination of pregnancy was established by the inspection of the animal.

Either it showed blood at the vulva or a loss of weight. In these two cases abortion can be regarded as having started. Some of the animals were killed immediately, others were opened 1 to 3 days later in order to have different stages of abortion.

TABLE I. EFFECT OF OVARIECTOMY ON THE PREGNANCY

No. of hamster	Stage of pregnancy, days	Time of abortion after ovariectomy, days	Results
H 7	About 10	3	Killed 1 day after abortion; partial retention of placenta
H 16	About 10	.	Killed 3 days after ovariectomy; complete abortion; eight sites visible but uterus empty
H 18	13	1	Killed the same day as abortion was observed; five sites; uterus empty
H 14	11	2	Killed 3 days after abortion; partial retention of placentae
H 37	10	2	Killed 2 days after abortion; sites visible; uterus empty
H 31	9	1	Killed 1 day after abortion; one uterine horn was pregnant, filled with blood and placenta
H 213	11	1	Killed 1 day after abortion; one dead foetus remains in the vagina
H 214	10	2	Killed 1 day after abortion; two foetuses in the vagina; one foetus inserted; three other sites empty

We do not know either for the hamster or for other rodents what is the exact mechanism of abortion after ovariectomy, but we have obvious signs of the endocrine disturbance produced by the removal of the ovaries. A most striking test is the transformation of the vaginal epithelium which takes place even before the embryos are aborted. As early as 24 hr. after ovariectomy, a massive excretion of mucus occurs. The cells rapidly empty of mucus and become cylindrical, while the nuclei of the basal layer enlarge exactly as in the castrate (fig. 5, Plate 17).

Control experiments were performed on six pregnant hamsters in order to exclude the suspicion of operative trauma. At least one of the two ovaries was completely left after the operation. The results are summarized in Table II. All animals had a normal parturition at term.

TABLE II. CONTROL EXPERIMENTS

No. of hamster	Stage of pregnancy, days	Operation performed	Results
H 31	11	Unilateral ovariectomy	Normal parturition 4 days later
H 23	12	Bilateral laparotomy, ovaries exposed then put back into the abdomen	Normal parturition 3 days later
H 21	9	Unilateral ovariectomy	Normal parturition 7 days later
H 38	12	Removal of one pregnant horn	Killed 4 days later; one normal embryo just being expelled
H 74	12	Injection of a sublethal dose of avertin	Normal parturition 4 days later

(*b*) *Substitution of ovarian hormones after ovariectomy in the pregnant female*

We have shown that ovariectomy, unlike control operations, terminates pregnancy. Attempts were then made to maintain pregnancy after ovariectomy by means of injections of ovarian hormones and especially of corpus luteum hormone, as it has been done previously in other species, e.g. the rabbit (Allen and Corner 1930; Courrier and Kehl 1933; Courrier 1935; Portman 1935). The animals (Tables IIIA and IIIB) were chosen at about the tenth day of pregnancy. They were injected intramuscularly with oil solutions, generally a day before ovariectomy and then daily, and they were examined, palpated and weighed every day. As soon as obvious loss of weight was observed, or when blood appeared at the vulva, it was held that abortion had begun, and the animal was then killed. In the absence of those signs, the female was opened on the day normal parturition would have been due. The animals were injected with rather large amounts, up to 0·5 I.U. a day, of progesterone over 4–5 days. As is shown in Table IIIA, not a single one had a normal parturition. Nine of them aborted or retained the foetuses dead for several days. In one case the foetuses seemed to have progressed in development, but even they were dead when the animal was opened.

In short, we completely failed to maintain pregnancy in the ovariectomized female with fairly high doses of crystal line progesterone. Histological examination of the vagina showed that in spite of continued injections of progesterone after ovariectomy, the typical mucification of the epithelial cells was not maintained, even in the animals which had retained the foetuses. The epithelial cells are columnar, but empty of mucus, the nuclei of the basal layer are enlarged (fig. 6, Plate 17). This is typical of the beginning of involution as seen after simple ovariectomy. This shows that progesterone alone does not replace the endocrine action of the ovaries, since it is not able to maintain the mucification which is associated with the presence of functional corpora lutea. We then studied the causation of mucification by injecting castrated females with ovarian hormones, and it was found that only simultaneous injections of progesterone and oestrone were able to produce the full mucification of pregnancy, whilst progesterone alone and oestrone alone were absolutely unable to produce the effect (Klein 1937). This result agreed with similar experiments performed on the rat by Selye, Browne and Collip (1936). We inferred from those facts that we might expect better results in maintaining pregnancy in the castrated hamster by the injection of progesterone and oestrone simultaneously, in amounts giving full mucification in the castrate. Our experimental results seem to

TABLE III A. INJECTIONS OF PROGESTERONE ALONE AFTER OVARIECTOMY IN THE PREGNANT FEMALE

No. of hamster	Stage of pregnancy at ovariectomy, days	Substance and amount injected	Result
H 3	12	Progestin 0·2 c.c. p.d.	Abortion 2 days later
H 11	12	Progestin 0·2 c.c. p.d.	Abortion 2 days later
H 20	About 10	Progesterone 0·5 mg. p.d.	Killed 6 days later; unilateral pregnancy; one macerated embryo retained
H 72	11	Progesterone 0·5 mg. p.d.	Abortion 4 days later; killed 2 days after abortion; partial retention of placentae
H 73	About 11	Progesterone 0·5 mg. p.d.	Blood at the vulva after 4 days; killed 1 day later; foetuses developed, dead; one embryo and placenta in the vagina
H 75	About 11	Progesterone 0·5 mg. p.d.	Blood at the vulva after 3 days; killed 4 days later; placenta partially retained
H 86	10	Progesterone 0·5 mg. p.d.	Killed 7 days later; foetuses resorbing
H 90	10	Progesterone 0·5 mg. p.d.	Killed 5 days later; foetuses resorbing
H 91	8	Progesterone 0·5 mg. p.d.	Blood at the vulva after 5 days; killed the same day; blood in the uterus; partial resorption
H 95	11	Progesterone 0·5 mg. p.d.	Blood at the vulva after 3 days; killed the same day; three foetuses retained, macerated and partially resorbed

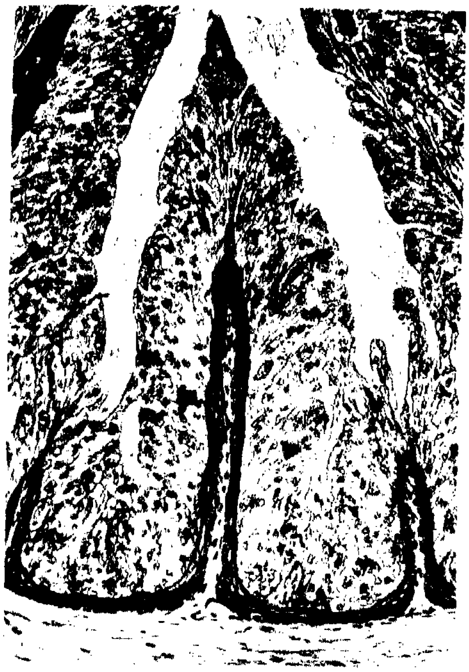


FIG. 1

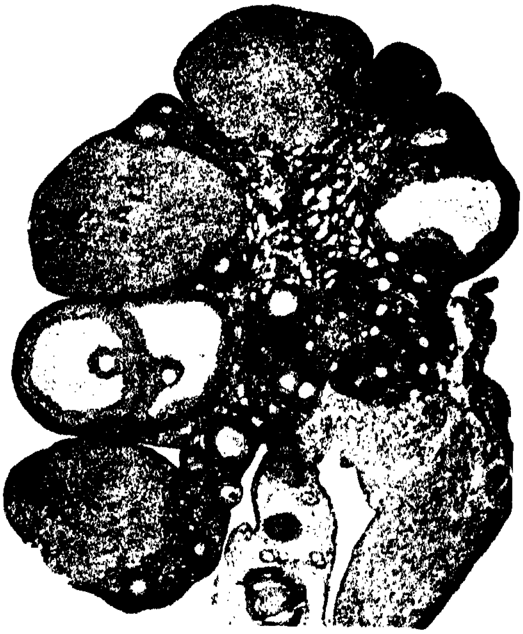


FIG. 4

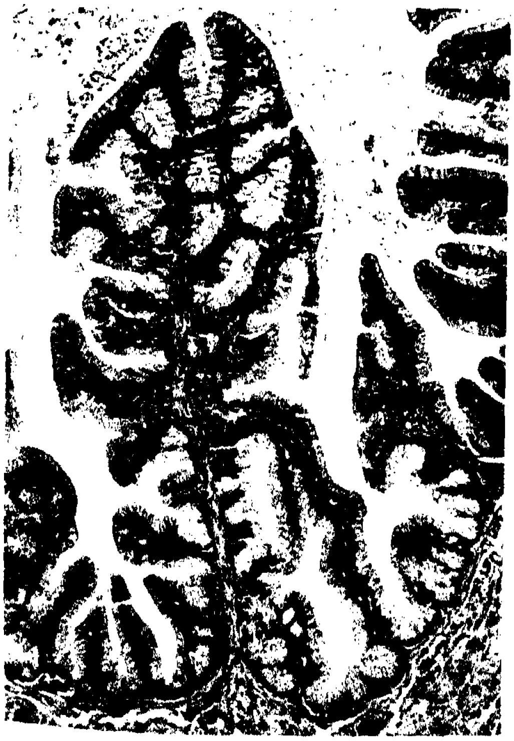


FIG. 2

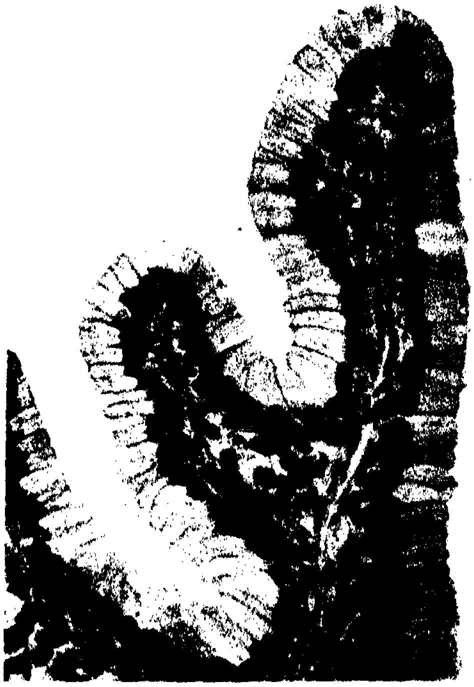


FIG. 3

(*Facing p.* 354)

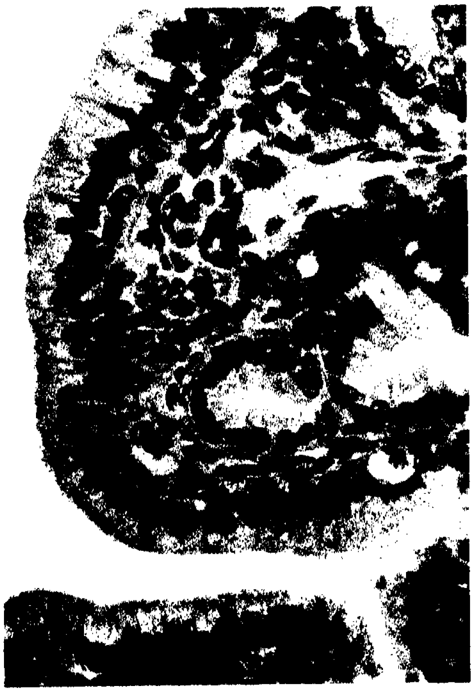


Fig. 5

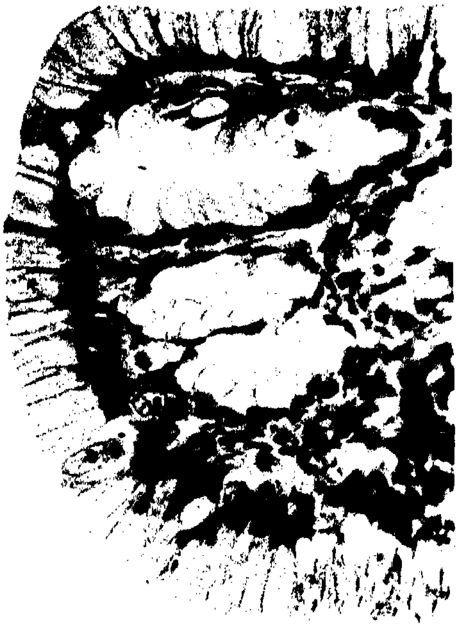


Fig. 7

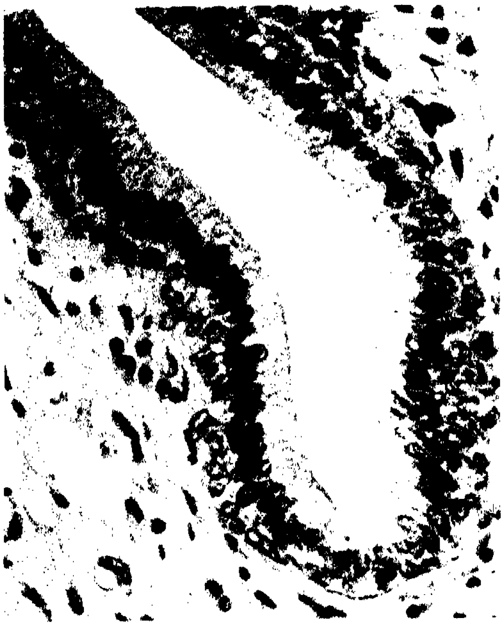


Fig. 6

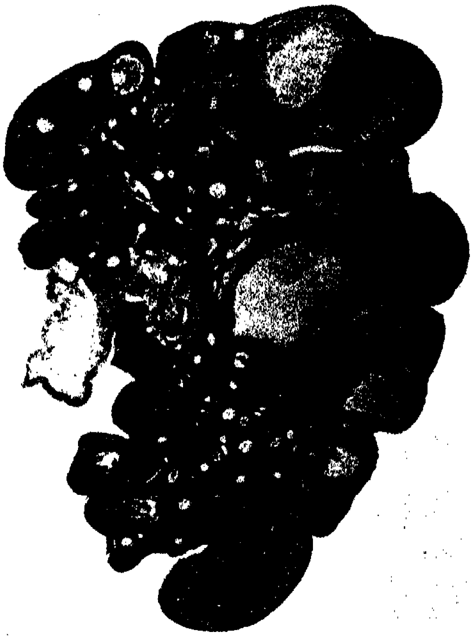


Fig. 8

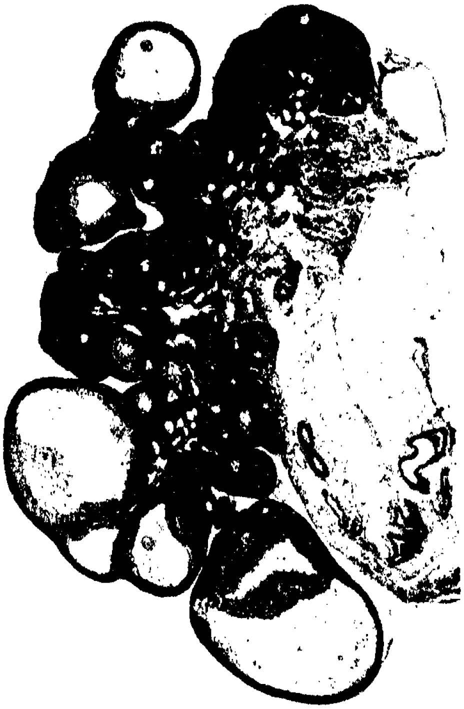


Fig. 9

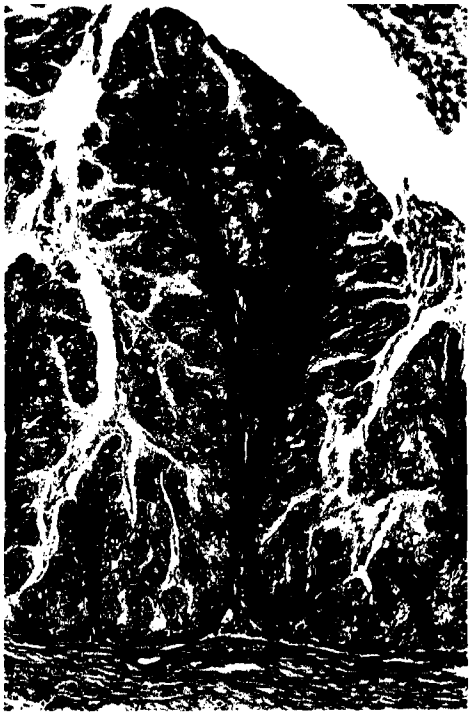


Fig. 10

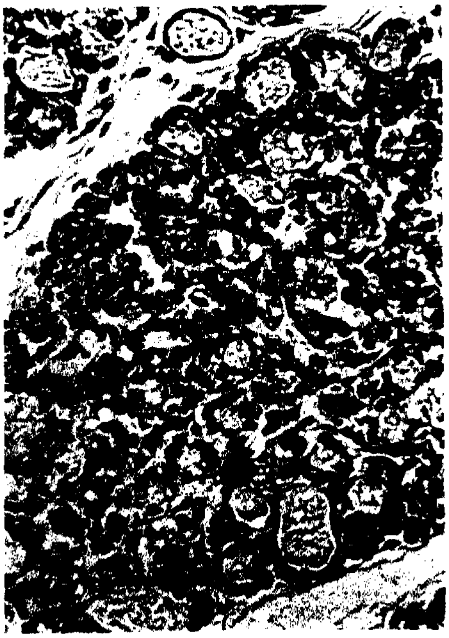


Fig. 12

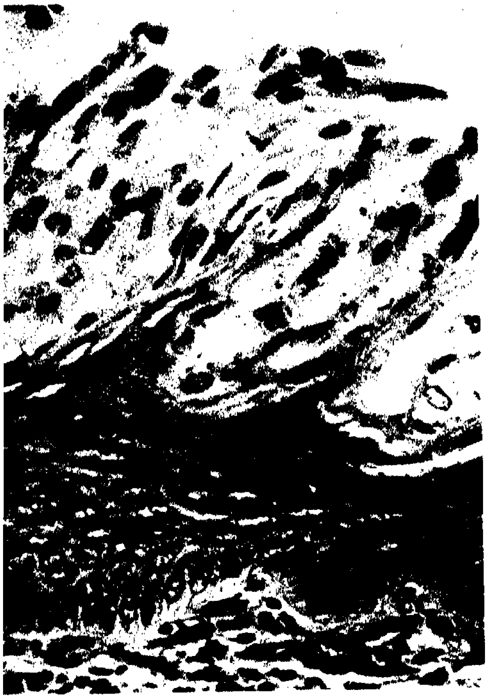


FIG. 11

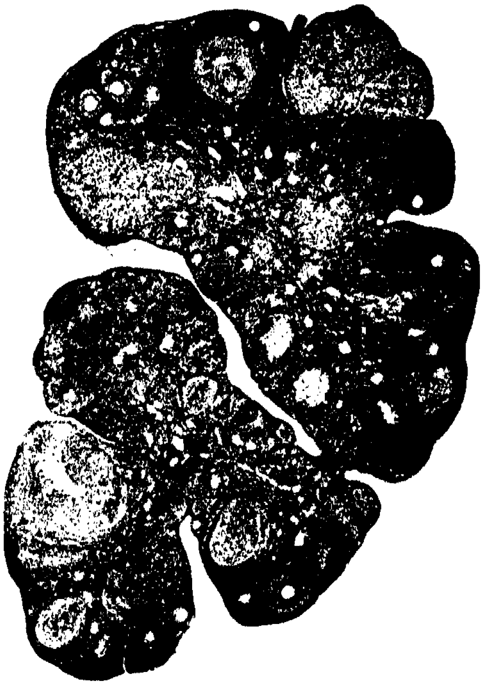


FIG. 14

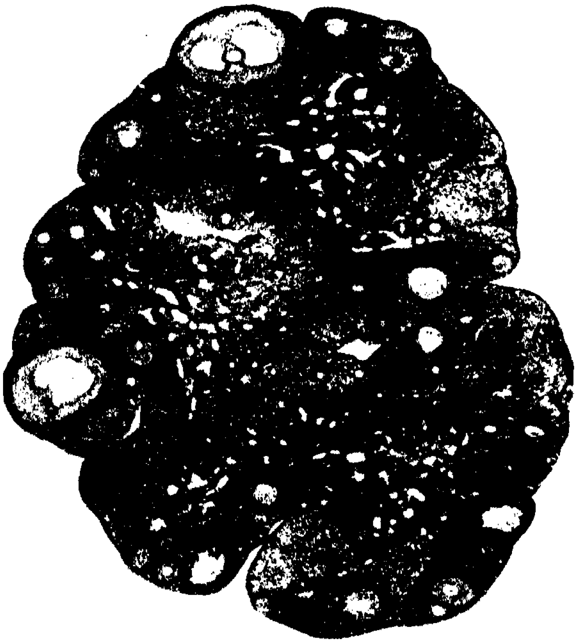


FIG. 13

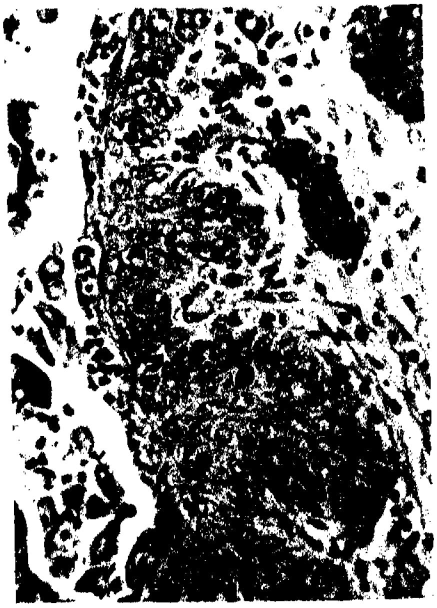


FIG. 15

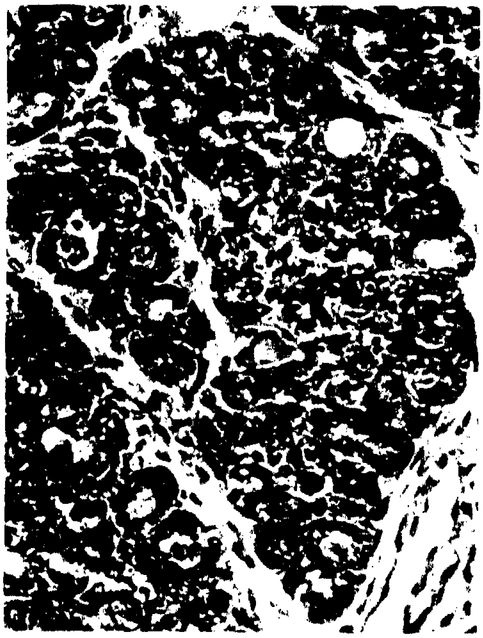


Fig. 16

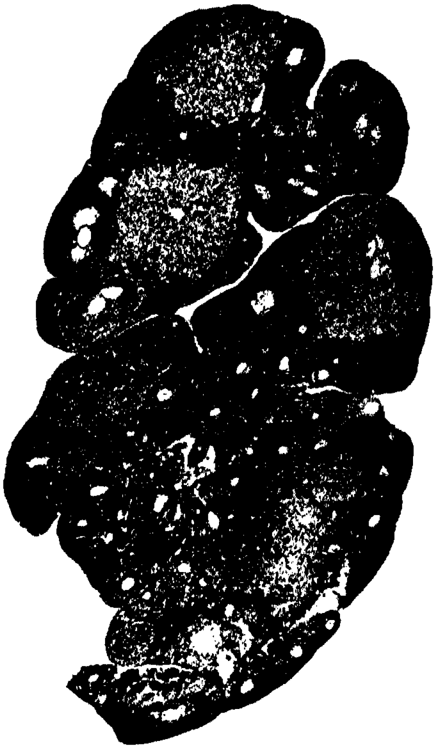


Fig. 17

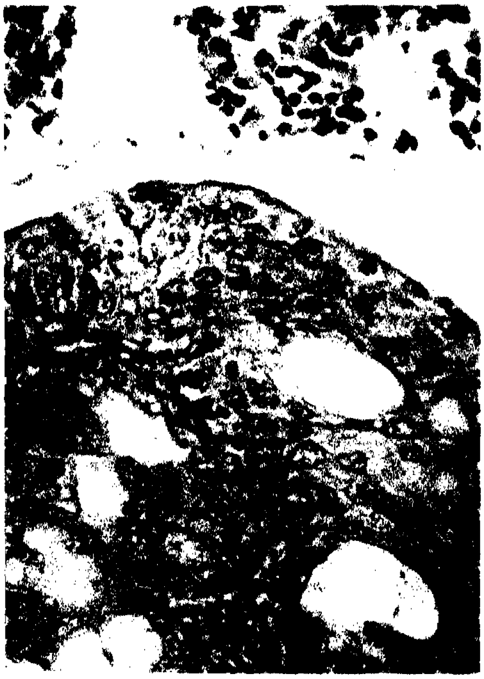


Fig. 18

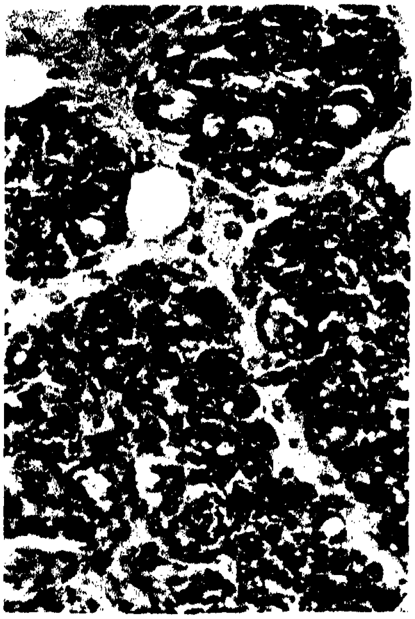


Fig. 19

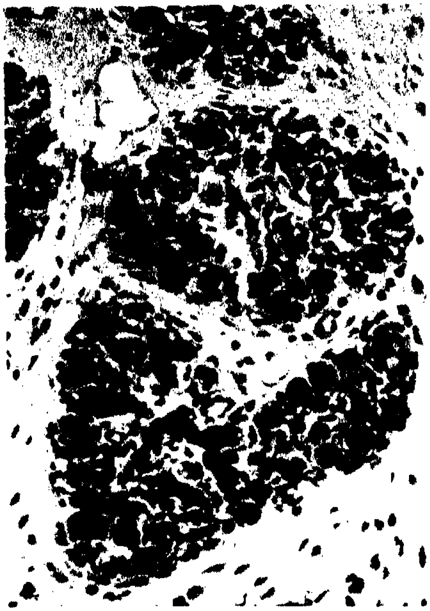


FIG. 20

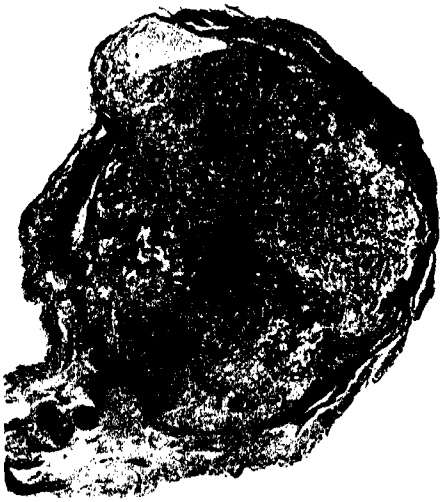


FIG. 21

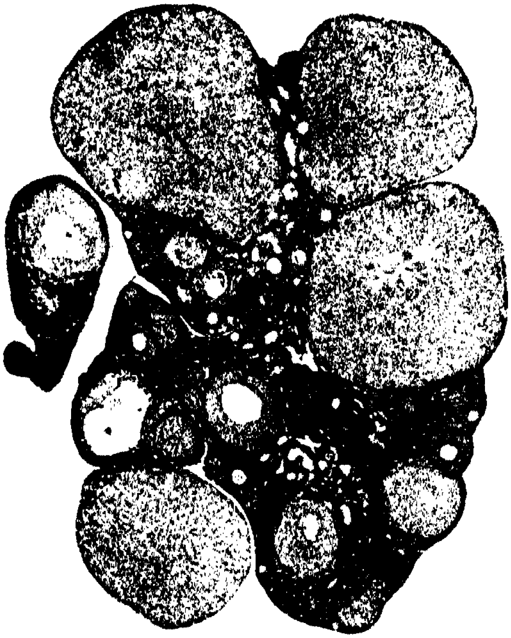


FIG. 22

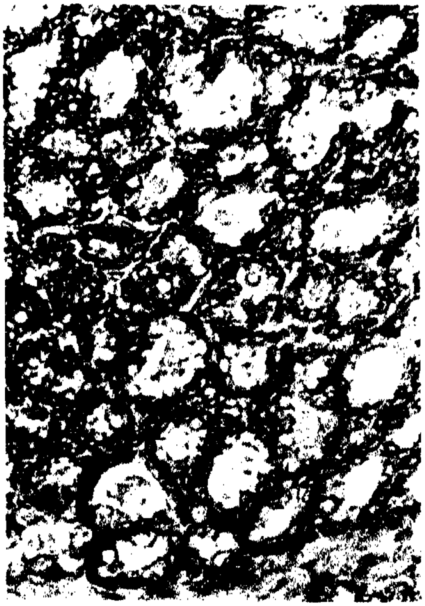


FIG. 24

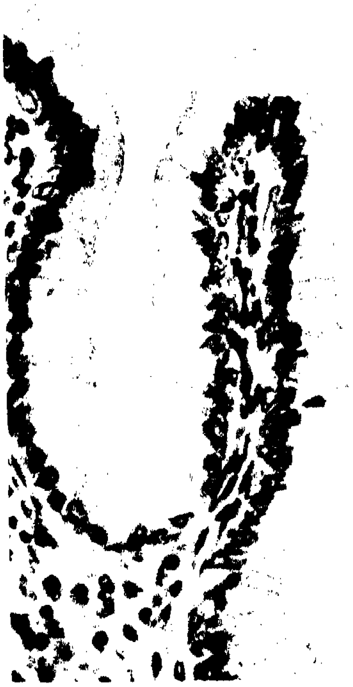
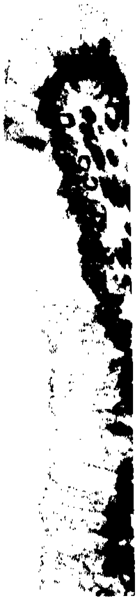


FIG. 23



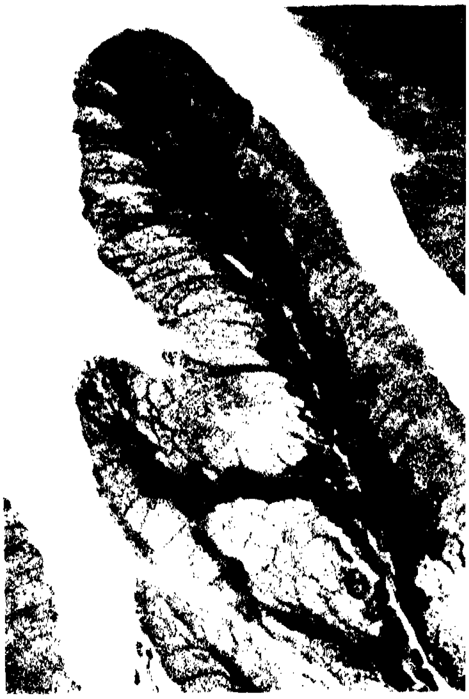


FIG. 25

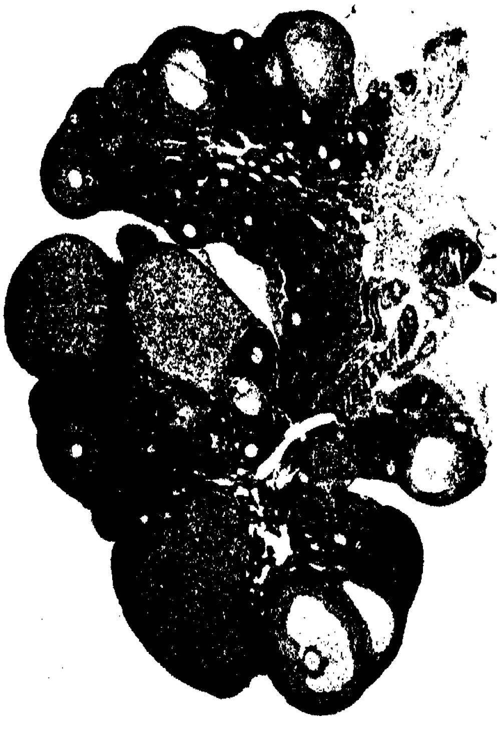


FIG. 26

confirm that idea (Table III B). Out of seven hamsters castrated at about the ninth day of pregnancy and injected simultaneously with progesterone and oestrone, not one aborted. Three animals had a normal parturition with living litters, which were born at term. In three other cases the foetuses were born at term, and were completely formed but dead. The last female died during parturition.

TABLE III B. SIMULTANEOUS INJECTIONS OF PROGESTERONE AND OESTRONE AFTER OVARIECTOMY IN THE PREGNANT FEMALE

No. of hamster	Stage of pregnancy at ovariectomy, days	Substances and amounts injected	Result
H 201	10	Progesterone 0·2 mg. p.d. Oestrone 1 γ p.d.	Blood at the vulva after 5 days, animal in a bad state, killed Eight foetuses completely formed; two in the vagina, all recently dead
H 206	11	as above	5 days later, *normal parturition*; the female suckles the young normally but only one is reared; animal killed a month later; genital organs of castrate type
H 207	9	as above	6 days later, *normal parturition*; the female suckles the three young normally, but 1 day later they are found dead; animal killed
H 208	About 12	as above	3 days later, blood at the vulva; animal in a bad state, killed; nine foetuses completely developed, one in the vagina
H 209	10	as above	5 days later, litter in the cage dead
H 210	10	as above	6 days later, *normal parturition*; three living young in the cage; 2 days later all dead
H 211	10	as above	5 days later, female dead in the cage; blood at the vulva; eight embryos at term in the cage; one embryo retained *in situ*

A histological section of the vagina the day the foetuses were born showed a unilayered cylindrical epithelium with tall mucified cells (fig. 7, Plate 17). The mucification was more pronounced than at normal parturition, when it always shows signs of regression. This fact indicates that although we had reproduced the endocrine conditions for the maintenance of pregnancy and vaginal mucification, we had not yet reproduced the exact conditions at normal parturition. In most cases, we diminished the amount of progesterone and raised that of oestrone in order to facilitate parturition, but we have not yet determined if this is necessary. On the other hand, it must be pointed out that the daily doses of progesterone were lower in this group of animals (Table III B) than in the foregoing (Table III A); so it remains to be determined whether the lower dose alone could not maintain pregnancy even in the absence of mucification.

We stopped the injections immediately at parturition if there were living litters. The females suckled them for 2 or 3 days, then let them die one after another except for one young which was reared. This shows that the ovarian hormones seem to be necessary for lactation, even though during that period there are no corpora lutea of lactation and the oestrone level seems to be very low (Deanesly 1938). Two of the animals which produced living litters were killed a month after parturition; the vagina showed complete castration regression, which demonstrates that ovariectomy had been complete.

These experiments show that progesterone alone injected into ovariectomized hamsters does not ensure the normal progress of pregnancy and does not maintain the typical mucification of the vagina. Progesterone injected simultaneously with oestrone into the ovariectomized female allows pregnancy to continue, and normal parturition may take place. The full mucification of the vaginal epithelium is maintained.*

6. CONNEXION BETWEEN THE PREGNANT UTERUS AND THE OVARIES

As we stated above, the corpus luteum of pseudopregnancy of the hamster lasts 7–10 days; the corpus luteum of pregnancy 16 days. In the foregoing groups of experiments we have seen that the corpus luteum controls the normal development of pregnancy until term. During the functional life of the corpora lutea the follicles do not mature, ovulation does not occur, the oestrous cycle is inhibited and the vagina shows the typical mucification which is a sign of simultaneous action of progesterone and oestrone. A new problem then arises. Why does the corpus luteum persist longer during pregnancy than it does outside gestation, and why is ovulation inhibited? A new element has appeared, that is the foetus with the placenta, the foetal membranes and the special uterine structures associated with the insertion of the embryo. Thus the gravid uterus may be suspected of having some influence on the ovary. We have dealt with this problem in previous experiments carried out on the rabbit and on the rat, and we have shown that the pregnant uterus controls the corpora lutea of pregnancy and the inhibition of the oestrous cycle (Klein 1933*a*, 1934, 1935*a*, *b*). We have carried out similar research on the hamster, in which species the corpora lutea disappear more rapidly and the ovaries undergo more obvious changes than in the rat and the rabbit.

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* In the hamster the histological study of the ovaries during pregnancy gives good evidence of the activity of the follicular apparatus. As has been stated by Deanesly and as the present author has noticed very often, beside the corpora lutea of pregnancy, numerous and large follicles are present during the whole pregnancy, and the theca of the follicles shows definite increase of the volume of the cells and hyperaemia. It seems therefore that, besides the functional corpora lutea, other glandular activity takes place in the ovaries (fig. 4, Plate 16).

The following experiments were performed in order to find evidence of a connexion between the pregnant uterus and the ovaries:

(*a*) Complete removal of the pregnant uterus, the ovaries and the vagina being left in place.

(*b*) Removal of the foetuses, the placentae, the ovaries and the vagina being left in place.

(*c*) Removal of the pregnant uterus, the ovaries and the vagina being left in place and the animal injected with ovarian hormones.

(*a*) *Removal of the pregnant uterus*

Between the eighth and the thirteenth days of pregnancy, the female is opened by an anterior laparotomy, the two pregnant horns are ligatured and sectioned at the level of the tubes and of the cervix. The ovaries are not touched at all, nor is the vagina. The changes observed in these two organs after the operation vary according to the time elapsed between the hysterectomy and the autopsy. The results are summarized in Table IV.

78 hr. after removal of the pregnant uterus. Serial sections of the ovaries show a striking shrinkage of the corpora lutea of pregnancy. The lutein cells are diminished in volume and the whole gland is less vascularized (fig. 8, Plate 17; fig. 9, plate 18). The follicles are enlarged and there is hyperaemia in the theca, but it is difficult to say if they differ from the condition seen in the normal pregnancy (figs. 8, 9, and compare with fig. 4). The vaginal epithelium has undergone important changes (fig. 10, Plate 18; fig. 11, Plate 19). The mucified cells lose their form and their regular arrangement and cluster into tufts which are shed into the lumen. The proliferating basal layer has formed a stratified malpighian epithelium the superficial cells of which are cornified (fig. 11). This cornification is occasionally more pronounced than in normal oestrus. The whole picture is similar to that of the foliaceous appearance which is typical for the normal oestrus in the hamster (compare figs. 10 and 1). Thus 14 days after mating and 78 hr. after the removal of the uterus the oestrous condition has superseded the unilayered columnar mucification typical of this stage of normal pregnancy (compare figs. 10, 11 with figs. 2, 3). The mammary gland shows a definite transformation too. A great number of acini are closing (fig. 12, Plate 18).

TABLE IV. REMOVAL OF THE PREGNANT UTERUS, OVARIES BEING LEFT

No. of hamster	Stage of pregnancy, days	Time of autopsy after hysterectomy	Results
H 15	8	4 days	Post-oestrus condition of the vagina
H 32	9	3 days	Foliaceous appearance of the vagina, oestrus
H 33	12	3 days	As H 32
H 23	11	6 days	Epithelium of the vagina thick with cysts, new corpora lutea in the ovaries
H 41	10	5 days	Vaginal epithelium elongated, typical of the beginning of the luteal phase
H 70	6	5 days	Vaginal epithelium pseudostratified
H 77	11	42 hr.	No change in the vagina; cells tall, cylindrical, mucified
H 83	12	68 hr.	Foliaceous appearance in the vagina
H 80	11	92 hr.	Vaginal epithelium stratified with polymorphs, in the lumen cornified cells and polymorphs; in the ovaries corpora lutea of pregnancy shrunken; a set of newly formed corpora lutea
H 81	11	140 hr.	Vaginal epithelium stratified, thick with cysts; corpora lutea of pregnancy nearly disappeared, a new set of cyclic corpora lutea
H 99	11	78 hr.	Foliaceous appearance of the vagina; corpora lutea of pregnancy show shrinkage
H 100	12	92 hr.	Foliaceous appearance of the vagina; corpora lutea of pregnancy nearly disappeared
H 105	13	72 hr.	Foliaceous appearance of the vagina; involution of the corpora lutea

92 *hr. after removal of the pregnant uterus.* There is greater shrinkage of the corpora lutea of pregnancy which have sunk into the ovarian tissue and no longer bulge on the surface of the ovary (fig. 13, Plate 19). The cells are small, the capillaries have disappeared, and these corpora lutea are no longer functional. But a set of new corpora lutea has appeared, easy to recognize by the cell shape, the vascularisation, the central cavity (fig. 14, Plate 19) and sometimes by the presence of the ovulation point. Corresponding changes have occurred in the vaginal epithelium. The mucified layers have disappeared except for remains mixed with cornified cells desquamated from the stratified epithelium; the latter is no longer cornified, and numerous polymorphs are migrating through into the lumen (fig. 15, Plate 19). This is a

typical metaoestrus picture. In the mammary gland more acini are closed (fig. 16, Plate 20).

140 *hr. after removal of the pregnant uterus.* The corpora lutea of pregnancy are reduced to some inconspicuous patches of cells. The newly formed corpora lutea have enlarged and they are almost solid, and they are well provided with capillaries. They are definitely smaller than the normal corpora lutea of pregnancy (fig. 17, Plate 20). The vaginal epithelium (fig. 18, Plate 20) is identical with that found in the normal luteal phase in the unmated female. In the mammary gland all acini with few exceptions are closed and form irregular masses of cells (figs. 19, Plate 20; fig. 20, Plate 21).

In short, the removal of the pregnant uterus between the eighth and the thirteenth days produces immediate degeneration of the corpora lutea of pregnancy. The ovarian cycle is no longer inhibited and recommences, with changes in the ovaries, and consequently in the vagina, identical with those described in the reproductive cycle of the normal hamster (see above and Deanesly 1938).

From the facts observed one may postulate the existence of a substance coming from the gravid uterus, which maintains the corpora lutea of pregnancy, produces inhibition of the ovarian cycle and thus takes part in the endocrine control of pregnancy.

(*b*) *Removal of the embryos*

In order to exclude the influence of the embryo and to locate more exactly the origin of the uterine factor responsible for the maintenance of the corpora lutea of pregnancy, we have carried out the removal of the foetuses, leaving the placentae, as done first by Weymeersch (1912). We have repeated it in the rabbit (Klein 1933*b*, 1934) and in the rat (1935*a*, *b*). It has been done too in the rat by Selye, Collip and Thomson (1934, 1935), in the guinea-pig by Nelson (1934), and in the mouse, in a different manner, by Newton (1935).

The animal is opened by an anterior laparotomy, the uterus is exposed, the swellings are incised one by one opposite the insertion of the placenta and the embryos removed. The ovaries are left in place. This operation was performed between the ninth and the twelfth days of pregnancy and the animals killed 4 days after (Table V).

At autopsy the placentae remain inserted (fig. 21, Plate 21). The ovaries show large well vascularized corpora lutea, and the ovaries in section are identical with those of a normal pregnancy (fig. 22, Plate 21, compare with fig. 4). Beside the corpora lutea, follicles of different sizes may be observed. The epithelium of the vagina is typical of pregnancy, being unilayered, with tall cylindrical, mucified cells (fig. 23, Plate 22, compare with fig. 3). It is clear, therefore, that progesterone, together with oestrone, is still present in the organism. The mammary gland is normal (fig. 24, Plate 21).

TABLE V. REMOVAL OF THE FOETUSES

No. of hamster	Stage of pregnancy, days	Time of autopsy after operation, days	Results
H 88	11	5	Placentae loose; vaginal mucification disappeared
H 96	12	3	Placentae remained inserted; vaginal mucification maintained; large florid corpora lutea of pregnancy in the ovary
H 101	9	4	Like H 96
H 103	10	4	Like H 96 and H 101

Thus, if the placentae remain intact, endocrine conditions normal for pregnancy are maintained, and it seems possible, therefore, to postulate that the placentae supply the stimulus which maintains the corpora lutea and thence produces inhibition of the oestrous cycle.

(c) *Removal of the pregnant uterus and injections of ovarian hormones*

By what mechanism does the placenta act on the ovaries? A possible endocrine activity of the placenta on the ovary has to be considered. Since Halban (1904) postulated an endocrine secretion of the placenta, much work has been carried out, especially during the last few years (see the reviews by Klein 1934; Brouha and Collin 1935; Gros 1936; Haterius 1935). The main conclusions at present are that the production of hormones by the placenta is extremely variable in quality and quantity in the different species, and that it is not possible to argue from the placenta of one species to that of another. Unfortunately to extract hamster placentae is at present impracticable.

Since recent work has indicated that the placenta may produce progesterone (Selye, Collip and Thomson 1935; Haterius 1935; Courrier and Gros 1935, 1936; Gros 1936) we have tried to replace the placenta by injections of progesterone and of oestrone, and to reproduce the special effect on the ovary of the placenta *in situ*. Table VI summarizes those experiments. Progesterone in amounts of 0·1–0·2 mg. a day is able to maintain the complete mucification for several days after the removal of the pregnant uterus, the ovaries being left in place (fig. 25, Plate 22), but the corpora lutea of pregnancy undergo regression just as if nothing had been injected after hysterectomy (fig. 26, Plate 22). Thus we conclude that the injected progesterone acts with the ovarian oestrogen in the maintenance of the vaginal mucification, but is incapable of maintaining the corpora lutea of pregnancy. Oestrone injected in the same conditions did not maintain the corpora lutea; in one case with small amounts of oestrone (H 84, Table VI) the mucification was partially maintained in the crypts, as is seen in similar experiments in castrates (Klein 1937), but the corpora lutea were markedly regressed. Thus neither of the two ovarian hormones seems able to replace the placenta in its action on the ovaries.

TABLE VI. REMOVAL OF THE PREGNANT UTERUS; OVARIES BEING LEFT. INJECTIONS OF OVARIAN HORMONES

No. of hamster	Stage of pregnancy, days	Product injected	Time of autopsy after hysterectomy	Results
H 56	8	Progesterone 0·2 mg. p.d.	5 days	Full mucification of the vagina; corpora lutea of pregnancy regressed
H 89	13	Progesterone 0·25 mg. p.d.	5 days	Mucification not maintained
H 97	11	Progesterone 0·2 mg. p.d.	78 hr.	Mucification maintained; corpora lutea in regression
H 98	13	Progesterone 0·2 mg. p.d.	92 hr.	Mucification maintained
H 107	10	Progesterone 0·15 mg. p.d.	5 days	Mucification maintained; corpora lutea of pregnancy regressed
H 109	13	Progesterone 0·1 mg. p.d.	3 days	Mucification maintained
H 71	12	Oestrone 2γ p.d.	6 days	Epithelium of the vagina stratified
H 84	13	Oestrone $0{\cdot}5\gamma$ p.d.	4 days	Mucification partially maintained in the crypts of the vaginal epithelium; corpora lutea of pregnancy markedly regressed

It must be emphasized that the placenta has to remain inserted in order to control the ovaries. We have previously observed on the rabbit (Klein 1933*b*, 1934) and on the rat (Klein 1935*a*, *b*) that as soon as the placentae become detached from the uterine wall after the removal of the embryos, all happens as if the whole uterus had been taken out, even if the placentae remain free in the uterus. We have observed one case of this type in the hamster (H. 88, Table V). An interaction between the placenta and the uterus walls may thus be necessary. Possibly a nervous stimulus is involved. Experiments on mechanical stimulation of the uterus determining the maintenance of the corpora lutea, like those of Long and

Evans (1922) on the rat, or those of Selye, Collip and Thomson (1934) support this view.

I wish to express my best thanks to Sir Henry Dale, F.R.S., and to the Medical Research Council for the hospitality of the National Institute for Medical Research, London, 1935–6.

It is with pleasure that I acknowledge my debt of thanks to Dr A. S. Parkes, F.R.S., and Dr Ruth Deanesly, for constant advice during the course of these experiments and for helpful assistance with the preparation of the manuscript. I am also most grateful to Dr Schoeller of Schering-Kahlbaum A.G., who generously supplied synthetic crystalline progesterone.

7. Summary

1. Experiments on the physiology of pregnancy have been carried out on the golden hamster.
2. Complete ovariectomy performed between the ninth and the thirteenth days results in the termination of pregnancy.
3. The injection of progesterone alone failed to maintain pregnancy and the typical vaginal mucification in hamsters ovariectomized about the tenth day after mating. The simultaneous injection of progesterone and oestrone did, however, maintain pregnancy and vaginal mucification in ovariectomized females, and in several cases we have obtained parturition at term with living litters. These results suggest that in the hamster, progesterone and oestrone may exercise endocrine control of pregnancy.
4. Complete removal of the pregnant uterus between the eighth and the thirteenth days causes the premature and rapid regression of the corpora lutea of pregnancy, and the ovarian cycle restarts. When the foetuses only are removed, by Caesarian section, and the placentae remain inserted, the corpora lutea of pregnancy are maintained and inhibition of the ovarian cycle continues.
5. These results agree with and complete our previous experiments on the rabbit and the rat. They show that there is a close correlation between the pregnant uterus and the ovaries, though we cannot yet postulate a satisfactory interpretation of the mechanism of the connexion.

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DESCRIPTION OF PLATES 16–22

All preparations are of the golden hamster

FIG. 1. Vaginal mucosa during oestrus. Foliaceous appearance of the epithelium. (Preparation by Dr R. Deanesly.) × 100.

FIG. 2. Vaginal mucosa on the tenth day of pregnancy. Epithelium unilayered, columnar, completely mucified. × 100.

FIG. 3. Same preparation as fig. 2. Tall, mucified epithelial cells. Here and there the nuclei of the basal layer are visible. × 400.

FIG. 4. Ovary on the tenth day of pregnancy. Three corpora lutea of pregnancy and follicles of different sizes. × 30.

FIG. 5. Vaginal mucosa of H 16, 3 days after ovariectomy performed on the tenth day of pregnancy. Abortion has taken place. The epithelial cells are still high, but quite empty of mucus. The basal layer is very obvious as is usual after ovariectomy. × 400.

FIG. 6. Vaginal mucosa of H 95, ovariectomized on the eleventh day of pregnancy, injected daily with 0·5 mg. of progesterone and killed 3 days after operation. Abortion has taken place. The cells are cylindrical but quite empty of mucus. The basal layer is very obvious. Cf. figs. 5, 7. × 400.

FIG. 7. Vaginal mucosa of H 207, ovariectomized on the ninth day of pregnancy, then injected daily simultaneously with 0·2 mg. progesterone and 1 γ oestrone. Normal parturition has taken place on the sixteenth day. The animal was killed 1 day after. Epithelium completely mucified. Cf. figs. 1, 6. × 400.

Figs. 8 and 9. Ovaries of H 99, 78 hr. after removal of the pregnant uterus on the 11th day of pregnancy. Regression of the corpora lutea; follicles of different sizes. Cf. figs. 4, 22. × 30.

Fig. 10. Vaginal mucosa of H 33, 3 days after removal of the pregnant uterus on the twelfth day of pregnancy. Foliaceous appearance of the epithelium. Cf. figs. 1, 2. × 100.

Fig. 11. Vaginal mucosa of H 99 (see figs. 8, 9). The layers of mucified cells are shed off into the lumen. Beneath is malpighian cornified epithelium. Cf. figs. 3, 23. × 400.

Fig. 12. Mammary gland of H 99 (see figs. 8, 9). Numerous acini closing. × 280.

Fig. 13. Ovary of H 100, 92 hr. after the removal of the pregnant uterus on the twelfth day of pregnancy. Marked regression of the corpora lutea of pregnancy. Cf. figs. 4, 22. × 30.

Fig. 14. Ovary of H 80, 92 hr. after removal of the pregnant uterus on the 11th day of pregnancy. Two regressed corpora lutea of pregnancy and one newly formed corpus luteum are visible. Cf. figs. 4, 22. × 30.

Fig. 15. Vaginal mucosa of H 80 (see fig. 14). Epithelium polystratified; polymorphs in the epithelium and in the lumen. A few cornified cells in the lumen. Cf. figs. 11, 3. × 400.

Fig. 16. Mammary gland of H 80 (see fig. 14). More acini closed than in fig. 12. × 280.

Fig. 17. Ovary of H 81, 140 hr. after removal of the pregnant uterus on the eleventh day of pregnancy. Some patches of the corpora lutea of pregnancy remain, three newly formed corpora lutea are visible. × 30.

Fig. 18. Vaginal mucosa of H 81 (see fig. 17). Epithelium stratified, thick, with cystic intercellular spaces. Polymorphs and cornified cells in the lumen. Cf. figs. 3, 11 and 15. × 400.

Fig. 19. Mammary gland of H 81 (see fig. 17). Nearly all acini are closed. Cf. figs. 12, 16. × 280.

Fig. 20. Mammary gland of H 23, 6 days after removal of the pregnant uterus on the eleventh day. All acini are closed. Cf. figs. 16, 19 and 24. × 280.

Fig. 21. Placenta of H 101, remaining inserted 4 days after removal of the foetuses on the ninth day of pregnancy. × 12.

Fig. 22. Ovary of H 101 (see fig. 21). Four vascular corpora lutea of pregnancy and follicles of medium size. Cf. figs. 4, 13 and 14. × 30.

Fig. 23. Vaginal mucosa of H 101 (see fig. 21). Epithelium unilayered; tall cylindrical cells, completely mucified. Cf. figs. 3, 11 and 15. × 400.

Fig. 24. Mammary gland of H 103, 4 days after removal of the foetuses, the placenta remaining inserted. All acini are open. Cf. fig. 16. × 280.

Fig. 25. Vaginal mucosa of H 97. Removal of the pregnant uterus on the eleventh day, then injected for 3 days with 0·2 mg. of progesterone. Mucification maintained. Cf. figs. 11, 23. × 400.

Fig. 26. Ovary of H 97 (see fig. 25). Three corpora lutea of pregnancy in regression. Cf. figs. 8, 9 and 22. × 30.

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Studies on the nature of the amphibian organization centre

VII. Evocation by some further chemical compounds

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[Plate 23]

Introduction

In a previous paper (Waddington and Needham 1935) evidence was presented that 1:9:dimethylphenanthrene, 9:10-dihydroxy-9:10-di-*n*-butyl-9:10-dihydro-1:2:5:6-dibenzanthracene and probably 1:2:5:6-dibenzanthracene are capable of evocating the formation of neutral tissue from the competent ectoderm of the newt gastrula. This paper records further tests with allied substances.

The work was done during the spring of 1937, with eggs of *Triton alpestris*, specimens of which were sent from Germany. The substance to be tested was inserted into the blastocoel of the young gastrula in a finely dispersed form contained in a lump of coagulated egg albumen. The technique of preparation of the coagula differed slightly from that previously employed. About 1 mg. of the substance was first dissolved in about 1 c.c. of acetone, the acetone solution added to 1 c.c. of water, and the acetone removed on a water-bath. About 1 c.c. of albumen solution, made in Holtfreter solution, was then added to the milky suspension, and the albumen coagulated on a water-bath. The dispersed substance seems to be almost completely adsorbed on the solid coagulum, giving a concentration of about 2 mg./c.c. The size of the implanted lumps was about 0·01 c.c., so that an embryo weighing roughly 5 mg. wet weight would receive about 0·02 mg. of the substance.

No attempt was made to give accurate doses; indeed, a technique for doing this would probably be rather difficult to develop. It was hoped that substances would either show a clear evocating capacity or entirely lack it.

This was, however, not the case, for the experimental results showed a gradation in evocating power. In the absence of exact dosing, a precise estimate of the relative efficacy of the different substances cannot be given, but the doses were all roughly the same, with a few exceptions noted below, and the approximate estimate of evocating power which is arrived at later in the paper is probably not very unreliable.

EXPERIMENTAL RESULTS

(a) *The controls*

In previous years, embryos operated in England have shown a considerable mortality, and the number of clear and satisfactory inductions has been low. It was therefore necessary to reinforce the evidence of the definite inductions by that of partial inductions, which produce only "neuralized thickenings" and not neural tubes. In 1937, conditions were very much better. Mortality was much lower, and a higher proportion of the eggs laid were fertilized and healthy. The egg-laying season also lasted longer into the summer. More important still, the reactivity of the embryos was on an altogether higher level, and a large proportion of inductions yielded well-formed neural tubes. Correlated with this, the reaction to the albumen which was used as an implantation medium was more extensive. A large number of control implantations were therefore made, both with albumen coagulated by heating in a watery solution, and albumen coagulated in water to which acetone had been added and evaporated off, as was done in the preparation of the dispersions of the tested substances.

No induced neural tubes were found in the controls, but very considerable ectodermal thickenings were produced in some cases. The ectoderm of these thickenings often showed some signs of "neuralization"; that is to say, the cells and nuclei might be elongated and arranged to form a palisade. These palisades were nearer to neural tissue than any which had been found in the controls of previous years, and nearer than those described by Wehmeier (1934). They would be reckoned as B+, and a few even perhaps as B++ on the previous classification (Needham, Waddington and Needham 1934); but there was, of course, never any difficulty in distinguishing them from true neural tubes.

It is clear that this year, with eggs showing this heightened reactivity, these partial inductions cannot be accepted even as corroborative evidence. The table of results (Table I) therefore records only the fully differentiated induced neural tubes or neural plates (category A of the previous classification). On the other hand, there is no reason to conclude that the results obtained with the highly sensitive eggs of this year make it necessary to disregard the evidence of the neuroid inductions obtained with the less reactive eggs of previous years. But even if this overcautious course is adopted, very few, if any, of the conclusions of the earlier papers would be affected, since they were nearly all based on the induction of definite neural tubes, to which the evidence of partial inductions was only confirmatory.

TABLE I

Substance	Series no.	No. tested	Inductions	Percentage inductions	Average vol. neural tissue in induction in 10^{-4} c.mm.
Styryl 430	F63*b*	12	8	66·6	143
Oestrone[*]	F52*b*	20	10	50	91
4 4′-Dihydroxy-diphenyl	F37*b*, F40*b*	8	2	25	44
1:2-Dihydroxy-1:2-di-α-naphthylacenaphthene	F44*b*	11	3	27	42
Methylcholanthrene	F58*b*	14	5	36	38
Sodium-1:2:5:6-dibenzanthracene-endo-α-β-succinate	F38*b*, F101*b*	26	10	39	35
Naphthalene (B.D.H.)	F98*b*	15	3	20	25
Cholesterol[†]	F76*b*	23	4	17	21
Pregnantriol	F64*b*	10	3	30	15
3:4-Benzpyrene[‡]	F57*b*	8	4	50	14
Pregnandiol	F59*b*	20	5	25	12
Anthracene	F72*b*	20	3	15	6
Squalene	F87*b*	14	1	7	5
5:6-*cyclo*-Penteno-1:2-benzanthracene	F71*b*	24	1	4	3
Sitosterol	F99*b*	17	1	6	3
Chrysene	F86*b*	17	0	0	0
Dihydrocholesterol	F66*b*	6	0	0	0
Equiline	F65*b*	11	0	0	0
Total implanted with substances		276	63	—	—
Controls:	F77*b*, F85*b*				
Albumen	F100*b*	58	0	0	0
Wax	F102*b*	10	0	0	0

[*] "Ketohydroxy oestrin."

[†] Free from ergosterol, oxycholesterol and cholestanol.

[‡] Formerly 1:2-benzpyrene.

(*b*) *The substances*

The substances tested and the results of the experiments are listed in Table I. The evocating power of the various substances is not truly reflected in the mere percentages in which neural tubes have appeared, since the induced tubes were of very different sizes in different specimens. As the implants were always of approximately the same size, a better indication of evocatory power can be given by a figure expressing the amount of neural tissue induced per implant. The volume of the induced tube was estimated by measuring its length (by the number of sections in which it appeared) and multiplying this by its height and width in a typical cross-section. This clearly results in an overestimate, since it assumes a rectangular cross-section and neglects the gradual narrowing of the tube towards its ends. Completely accurate figures could, however, only be obtained by laborious measurements of each section, and since the doses were not accurately measured, the approximate estimate arrived at by the above method is adequate for the rough comparison which is all that is in question here.

A few of the experiments require special mention. Two different methods were adopted in preparing the coagula containing styryl 430; many of them were made in the usual way, the styryl 430 being first dissolved in acetone and then added to water, but in others the styryl was dissolved in water in the first place, no acetone being used. The coagula in both instances were coloured a pale pink. The implants of sodium-1:2:5:6-dibenzanthracene-9:10-endo-α-β-succinate were similarly prepared from a water solution without the use of acetone. Finally the anthracene and naphthalene were used in rather higher concentrations than the other substances, probably about four times as high. The anthracene was a highly purified specimen, but the napthalene was the normal commercial material (B.D.H.) and therefore was undoubtedly less pure.

Discussion

The first point which emerges from Table I is that evocation cannot be a reaction to an unspecific stimulus. This is clear from the number of controls, which were made under the same conditions as the experiments. In the whole series of experiments involving the implantation of albumen containing dispersed substances, sixty-three inductions were obtained in 276 embryos; the percentage is nearly twenty-three. If induction were due to the mechanical stimulus of the implant, the chance that none would occur in the fifty-eight controls would be less than one in three million. It is perfectly clear, therefore, that the inductions are really reactions to the substances adsorbed on the albumen and not to the mere presence of a foreign body.

Most of the more potent inducing substances in Table I are already known to be active in other biological reactions. Methylcholanthrene and 1:2:5:6-dibenzanthracene are two of the most powerful carcinogens discovered by Kennaway and Cook, while styryl 430 is potent in the same way (Browning, Gulbranson and Niven 1936). Oestrone is oestrogenic, and so, though only in doses about 100,000 times as large, are 4:4-dihydroxydiphenyl and 1:2-dihydroxy-1:2-di-α-naphthyl-acenaphthene (Dodds and Lawson 1936). Naphthalene occupies an anomalous position, since three large inductions were obtained with it; the specimen used, however, was not specially purified, and the dose applied was greater than that of the other compounds tested, so that it is not unlikely that the inductions were produced by some impurity.

With the exception of naphthalene, none of the biologically inactive compounds which were tested showed any strong inducing capacity, though pregnandiol and pregnantriol were fairly active. Some activity was also shown by a highly purified specimen of cholesterol, free from ergosterol, oxycholesterol, and cholestanol. In a previous experiment involving nineteen embryos, no activity was found in B.D.H. cholesterol or residues obtained in its preparation (Waddington, Needham, Nowinski and Lemberg 1935); this may perhaps have been due to the low reactivity of the eggs in that year, or there may possibly have been some other related substance present in the preparation now used.

It is surprising to find that, though the biologically inactive substances do not show strong evocating power, they nearly all occasionally produce an induction. Thus we find one small induction produced by squalene and one by sitosterol, while there are as many as three small inductions performed by anthracene.

Although we may deduce from these facts that evocating power probably has some relation to oestrogenic and carcinogenic activity, it clearly does not run parallel to either of them. Thus 5:6-*cyclo*-penteno-1:2-benzanthracene is powerfully carcinogenic (Cook 1932) and has also some oestrogenic capacity (Cook, Dodds, Hewett and Lawson 1934) and equiline is oestrogenic; both compounds show very slight evocatory capacity. 3:4-Benzpyrene, one of the most potent carcinogens known, showed no activity in a series of implants performed in 1934 (Waddington and Needham 1935) and only a moderate degree in the present work with much more reactive eggs.

It would certainly not be profitable at the present stage to attempt to form any hypothesis as to the chemical basis of inducing power. Even as regards oestrogenic or carcinogenic activity, which is being investigated by many workers in different laboratories, the data do not yet allow of more than very tentative suggestions. Before any hypotheses can be put forward about inducing power, it will be necessary to investigate a much larger range of substances and also to work out a method of quantitative assay, a matter which will probably present considerable technical difficulties. Further, some method must be discovered of distinguishing between substances which are active because they are sufficiently similar to the natural evocator to stimulate the same reactive system in the ectoderm, and substances which act by releasing the evocator which is known to be already present but inactivated within the ectoderm. As yet we cannot tell in which of these ways a given substance is acting, and our only guide is the rather indecisive argument from dosage (Waddington, Needham and Brachet 1936; Needham 1936).

The inductions described in this paper by substances such as anthracene, squalene, etc. present a particular difficulty. It is not easy to believe that these substances act by a mechanism involving the breakdown of an inactive evocator complex in the way which has been suggested for methylene blue and acids. On the other hand, it may be difficult to suppose that the reactive system is so unselective that all these compounds can stimulate it; though the extraordinary non-specificity of the oestrous system warns us against taking any such argument too seriously. But in any case, there is a further mechanism which cannot be excluded from consideration. If, as seems probable, the inactivation of the evocator contained in the ectoderm is due to the formation of a loose compound with some other molecules, there is the possibility that substances which resemble the evocator only rather remotely may also have an affinity for the inactivating component of the evocator complex, and, by forming a complex compound themselves, may cause some of the evocator to be liberated. A mechanism of this kind may be at work with some of the substances tested here.

At the present time there is no way of testing such hypotheses, and it is hardly profitable to discuss them further. The basic difficulty remains that the only test we have for evocating power is to apply a substance to the ectoderm, in which the evocator is already contained. Until this difficulty can be surmounted it appears impossible to discover the true nature of the natural evocator by implanting synthetic substances. For this purpose a return must be made to the investigation of the chemical properties of evocating and non-evocating tissues from the embryo. The investigation of synthetic inducing substances remains an interesting and important problem, but, just as in the case of synthetic carcinogens, one

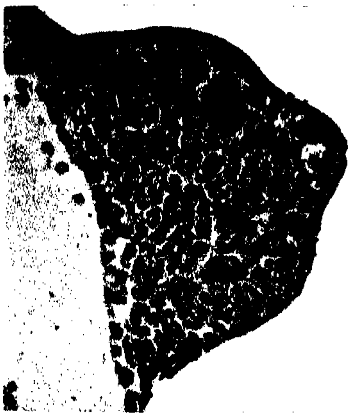

Fig. 1

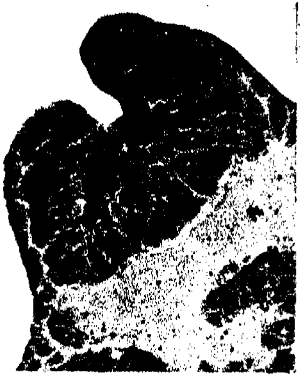

Fig. 2

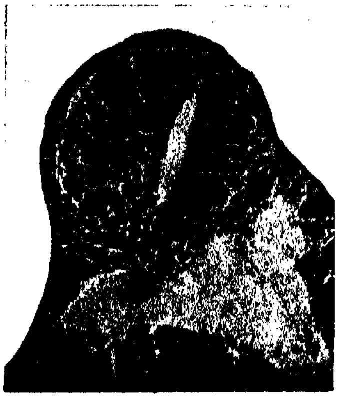

Fig. 3

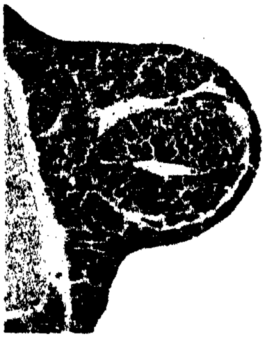

Fig. 4

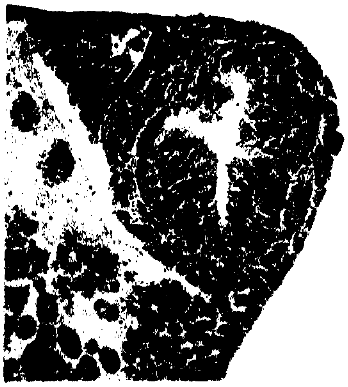

Fig. 5

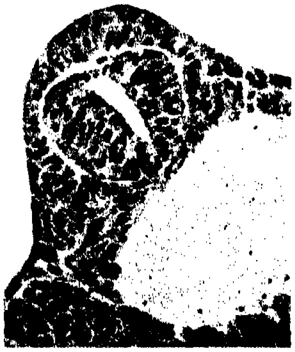

Fig. 6

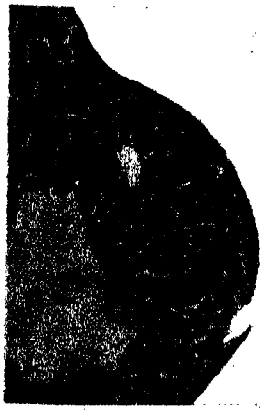

Fig. 7

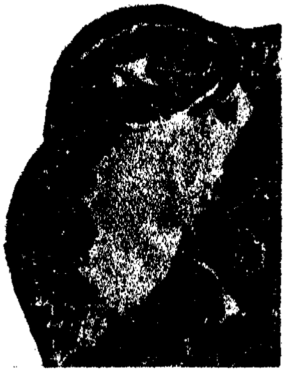

Fig. 8

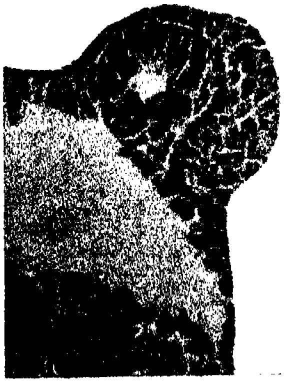

Fig. 9

must beware of assuming that the artificial process gives a true picture of what happens in nature.

ACKNOWLEDGEMENTS

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Dr E. Boyland for sodium 1:2:5:6-dibenzanthracene-endo-α-β-succinate and styryl 430; Dr J. W. Cook for methylcholanthrene, 3:4-benzpyrene, and 5:6-*cyclo*-penteno-1:2-benzanthracene; Professor E. C. Dodds for 4:4′-dihydroxy-diphenyl, and 1:2-dihydroxy-1:2-di-α-naphthyl-acenaphthene; Professor I. M. Heilbron for squalene; Dr G. Marrian of Montreal for equiline, pregnandiol and pregnantriol; Dr O. Rosenheim for oestrone, sitosterol, cholesterol, anthracene and chrysene; Dr R. Schönheimer of New York for dihydrocholesterol. I also wish to thank Dr J. Holtfreter of Munich, who arranged to supply me with *Triton alpestris*. Finally I wish to express my gratitude to the Rockefeller Foundation, which made a grant to defray the expenses of a histological technician.

SUMMARY

1. Certain carcinogenic and oestrogenic substances, and other related compounds, were tested for evocator capacity by implantation in the blastocoel of newt gastrulae.

2. The substances were implanted in the form of suspensions in coagulated egg albumen. In an extensive series of controls, no inductions were obtained with albumen alone.

3. In the experimental series, a large number of inductions were obtained, the eggs showing much greater reactivity than in previous years. The results are summarized in Table I.

4. Inductions were obtained with a considerable number of substances, and no hypothesis can yet be made as to the relation between chemical structure and evocator power.

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Description of Plate

Fig. 1. F100*b*–16. Control albumen. This was the largest thickening obtained. × 130.

Fig. 2. F52*b*–16. Oestrone, induced open neural plate. × 130.

Fig. 3. F53*b*–1. Oestrone, induced neural plate. × 130.

Fig. 4. F63*b*–6. Styryl 430, induced neural plate. × 130.

Fig. 5. F38*b*–5. Sodium-1 : 2 : 5 : 6-dibenzanthracene-endo-α-β-succinate, induced neural plate. × 130.

Fig. 6. F64*b*–7. Pregnantriol, induced neural plate. × 130.

Fig. 7. F59*b*–5. Pregnandiol, induced neural plate. × 130.

Fig. 8. F72*b*–3. Anthracene, induced neural plate. × 130.

Fig. 9. F99*b*–12. Sitosterol, induced neural plate. × 130.

576.314:581.823

The structure of the walls of parenchyma in *Avena* coleoptiles

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Introduction

The structure of the primary wall and its relation to that of the secondary wall has long been a subject of controversy. In recent years evidence collected from various sources has led to somewhat divergent views. Thus consideration of the development of the conifer tracheid, for instance, has led to the suggestion that the molecular chains of cellulose, in the wall of the cambial initial, are inclined rather steeply to the longitudinal axis, so that they form a spiral round the cell (Jaccard and Frey 1928; Preston 1934). On the other hand, a different idea may be derived from consideration of transverse sections of tracheids, as made by Bailey and Kerr (1935) and by Freudenberg and Dürr (1932) under the polarizing microscope. Observed in this way the tracheid wall clearly consists of at least three layers—an outer, and an inner, bright layer and a central dark layer. On the basis of such observations, Bailey and Kerr and Freudenberg have concluded that the direction of the cellulose micelles varies from point to point in the wall thickness. In the outer and inner layers they are said to run transversely and in the central layer longitudinally. This would confirm the earlier work of Scarth, Gibbs and Spier (1929). While the validity of this interpretation is open to question (as will be pointed out elsewhere) it would possibly imply that since the outer layers of the secondary wall lie nearest to the primary wall, the micelles in the latter would be oriented in the transverse plane rather than in a spiral. Certain suggestions concerning the relation between wall deposition and growth have, in fact, been made in terms of this transverse orientation in primary walls. Consideration of these hypotheses has been made elsewhere (Preston and Astbury 1937); the present paper is concerned more with a discussion of some evidence which has been quoted in their support (Castle 1937).

According to Bonner (1936) the walls of the parenchyma of oat coleoptiles contain cellulose chains which lie transversely in the wall, exactly as suggested for the primary walls of other tissues in the latter of the two alternatives mentioned above. It is quite clear that the primary walls, in some cases at least, contain cellulose (Tupper-Carey and Priestley 1923; Priestley and Tupper-Carey 1922; Bonner 1936; Kerr and Bailey 1934) and that the molecular chains of this polysaccharide may be arranged sufficiently parallel to give a good X-ray diagram (Sisson 1937). Double refraction phenomena, which were used by Bonner, are certainly to be explained in terms of the organization of the cellulose constituent of the wall.* While his paper contains much that is of interest and value, it is by no means obvious that the experimental technique employed in the study of the parenchyma wall is such as to allow Bonner's conclusions to be upheld. The maceration technique, in the first place, seems to be unnecessarily drastic, but more important is the fact that measurement of extinction positions was apparently made on whole cells, rather than on single walls. The major extinction position is found to lie transversely, and Bonner concludes that the molecular chains of cellulose are similarly oriented. This conclusion is not contained in the evidence; for the major extinction position of a whole cell (double wall) would clearly lie transversely whether those of the individual walls were transverse, or inclined as parts of a spiral making an angle greater than 45° to the longitudinal axis (fig. 1). In view of this ambiguity in the evidence available, it was considered necessary to re-examine the structure of the walls of these cells. The experimental technique as developed up to the moment is too tedious to allow the presentation of very numerous results. Such results as are put forward, however, are sufficient totally to contradict Bonner's suggestion; and they lead to an entirely different conception of the relation between cell extension and wall structure.

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* See footnote, p. 380.

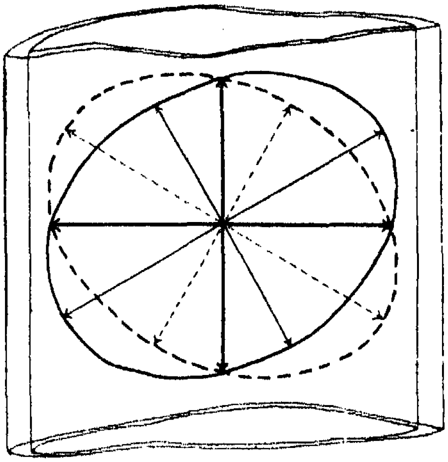

FIG. 1. Diagrammatic representation of the optical character of a cell with both front and back walls present. The major extinction position of the double wall bisects the acute angle between those of the individual walls. ←→ Extinction positions of front wall. ←···→ Extinction positions of back wall. ⟵⟶ Extinction positions of double wall. The longer lines represent major extinction positions, and the shorter lines minor.

The coleoptile may readily be recognized in the seed of *Avena* as a complete cylinder, closed at the apex by a blunt cone. It represents the first aerial leaf, and differs from later leaves in having no lamina. The whole organ, at this stage, consists of small cells, with dense cytoplasmic contents and thin walls—the so-called meristematic cells. On germination of the seed, this meristematic condition disappears and the coleoptile expands solely by vacuolation; cell division seems to play no part in the process (Tetley and Priestley 1927). Normally, the adult coleoptile is some 1·5 mm. in diameter and may attain a length between 1 and 6 cm. according to cultural conditions. An epidermal layer is found on the inner and outer faces of the hollow cylinder thus developed, while the inner tissues consist mainly of parenchyma, corresponding to the mesophyll of a more typical leaf. It is with these latter cells that the present paper is concerned. They may be distinguished by the optical characters of their walls (the outer wall of epidermal cells is more strongly birefringent than the walls of the parenchyma and the cellulose chains composing it appear to lie longitudinally) and by morphological features.

EXPERIMENTAL METHODS

A number of oat grains were germinated in damp sawdust under constant light conditions, at a temperature of some 25° C. Coleoptiles were removed as they reached a suitable size—some 25–35 mm. in length. Each coleoptile was treated independently by removing the apical 2 mm. and the basal 2 mm., together with a section 2 mm. in length from about the mid-point. The specimens thus obtained were immersed in 5 % chromic acid without previous fixation, and were not removed until maceration was complete. Each was then washed in distilled water, transferred individually to a slide previously treated with a thin coat of albumin fixative, and the tissue teased apart with needles. Subsequent drying and immersion in absolute alcohol served to attach the parenchyma very firmly to the slide. The method of dissection used in removing one wall of a conifer tracheid (Preston 1934), in which a microtome knife is passed over the slide, was found to be ineffective owing to the general collapse of the cells on drying. Cells were therefore treated singly by placing a drop of water on the specimen and scraping gently, under the microscope, either with a fine glass needle as used in micromanipulation work, or with the tungsten needles described by Bell and Facey (1937). The latter were found to be quite suitable, though a needle of sufficient fineness shows a pronounced tendency to bend plastically. Only those cells were subsequently studied from which a small piece of the upper wall was removed, allowing observation of a single wall, and of which the remaining portions of the (double) wall were sufficiently free from folds and wrinkles to permit a fairly accurate estimate of cell dimensions. The number of successful treatments was of necessity rather low (two or three per slide being an average figure) particularly in the case of the smaller cells. It was found impossible, in the batch of material prepared, to dissect without serious disintegration of the cell as a whole, any parenchyma from coleoptiles less than 25 mm. in length. Measurement of cell dimensions was carried out in the usual manner with the aid of an eyepiece micrometer. For determination of the direction of the major extinction position, a colour plate Blue II was employed, since this seemed to be the most sensitive of the plates available and made such measurements reasonably accurate, even of material apparently isotropic when viewed between crossed nicols alone. It is not proposed here to give any account of the principles involved in these measurements; they may readily be obtained from any text-book on the subject (e.g. Johannsen 1918; Winchell 1931; Ambronn and Frey 1926).

RESULTS

The following observations refer exclusively to the parenchyma of the coleoptile which, as has already been pointed out, may readily be distinguished from the epidermal cells, even in macerated material, both by its general morphological characters and by its optical properties. The parenchyma is relatively thin-walled and, as observed by Bonner, generally shows negative birefringence when the intact cell is observed. Yet there are interesting features in the relation of birefringence to the dimensions of the cell which have not hitherto been pointed out. In any coleoptile, the shorter and wider cells invariably possess negative birefringence (in the sense that the major extinction position lies transversely). As progressively longer or thinner cells are observed, however, the strength of birefringence gradually diminishes, until at a certain stage the double wall becomes isotropic. When the length increases beyond this value, or if the cell is inordinately narrow, the birefringence becomes positive; and from this point tends to increase with increasing cell length, or with decreasing diameter. In Table I are listed a number of determinations taken from intact cells of one coleoptile. No definite figures are given for the value of the birefringence, since in the majority of cases it falls below the limit of accurate measurement even with the Sénarmont compensator. The section "fairly strong" includes those cases in which the birefringence is just detectable between crossed nicols alone; and the remainder of the sections are graded according to the degree of colour change when the specimen is rotated over a colour plate Blue II. Although there is no exact correlation between either length or girth and the birefringence, for clearly it is a combination of both variables which is important, the general run of the observations is as described above.

Again, the major extinction position of the intact cell is often not quite transverse, and this parenchyma constitutes the only cells observed by the author in which the extinction position is inclined to the cell axes. The series of measurements presented in Table II will serve to show that this deviation from the transverse direction is often considerable, and in excess

TABLE I. PARENCHYMA FROM BASAL REGION OF A 35 MM. COLEOPTILE

Length mm.	Breadth mm.	Birefringence	
0·139	0·095	f.s.	Negative
0·228	0·125	f.s.	
0·174	0·105	w.	
0·195	0·081	w.	
0·239	0·127	w.	
0·256	0·054	w.	
0·261	0·065	w.	
0·272	0·118	w.	
0·292	0·034	v.w.	
0·306	0·061	v.w.	
0·253	0·039		Isotropic
0·442	0·042		
0·236	0·039	w.	Positive
0·278	0·033	w.	
0·306	0·046	w.	
0·306	0·037	f.s.	

f.s. = fairly strong; w. = weak; v.w. = very weak.

TABLE II. PARENCHYMA FROM A 35 MM. COLEOPTILE

Position of parenchyma	Dimensions		Inclination of major extinction position (double wall) to transverse direction
	Length	Breadth	
Base	0·195	0·102	− 6·5 ± 3
	0·233	0·093	− 15·0 ± 2
	0·466	0·087	+ 15·0 ± 2
	0·300	0·064	+ 15·0 ± 2
	0·344	0·106	− 17·5 ± 2
	0·225	0·075	+ 20·5 ± 5
Middle	0·334	0·063	+ 18·5 ± 2
	0·294	0·063	− 18·0 ± 1
	0·250	0·086	− 7·0 ± 2
	0·256	0·071	− 10·0 ± 3
	0·252	0·104	− 14·5 ± 2
	0·274	0·077	− 14·5 ± 2
	0·176	0·060	+ 10·5 ± 1
	0·167	0·060	+ 10·5 ± 4
	0·246	0·055	+ 8·5 ± 3

of the experimental error. Whatever this may mean in terms of cell development, it is clear that the extinction direction of one wall at least is not transverse, and it would seem that the probable explanation of this, and the previously described phenomena, is that the major extinction position of each wall is inclined. The wall must then be wound with a molecular spiral, and this latter observation would necessarily imply that two opposite regions of the parenchyma wall are not quite identical—there must be a difference in the direction of the extinction position, in the wall thickness, or in the structural arrangement of the cellulose itself.

TABLE III

Length of coleoptile (mm.) and position of parenchyma	Dimensions of cell		Inclination θ	$\frac{L}{b \cot \theta}$
	Length, L (mm.)	Breadth when flattened (mm.) ($b/2$)		
25 Base	0·315	0·036	r45·0 ± 1·5	4·37 ± 0·22
	0·223	0·076	l70·0 ± 5·0	2·62 ± 0·52
25 Middle	0·176	0·129	l75·5 ± 2·0	2·65 ± 0·42
35 Base	0·248	0·064	r30·0 ± 3·0	1·48 ± 0·45
	0·202	0·064	l60·0 ± 2·0	2·74 ± 0·21
	0·418	0·112	r65·0 ± 3·0	4·10 ± 0·56
	0·282	0·084	l56·0 ± 1·0	2·50 ± 0·09
	0·278	0·034	l30·0 ± 1·0	2·35 ± 0·09
	0·239	0·129	l58·0 ± 2·0	1·49 ± 0·12
35 Middle	0·150	0·059	r60·0 ± 1·0	2·22 ± 0·10
	0·222	0·078	r70·0 ± 2·0	3·93 ± 0·43
	0·234	0·046	r39·0 ± 1·0	2·04 ± 0·09
	0·222	0·081	l62·0 ± 2·0	2·59 ± 0·22
	0·258	0·079	r50·0 ± 3·0	1·95 ± 0·20

This type of explanation is fully confirmed when the structure of the single wall is considered. The major extinction position of such a wall is always inclined at a considerable angle to the transverse direction. This will be clear from Table III, where the inclinations refer to the angle between the major extinction position and the longitudinal axis of the cell. This latter axis is here defined as the bisector of the angle between the direction of the lateral walls. Undoubtedly, then, the evidence is in favour of a spiral configuration of cellulose chains and in direct contradiction to Bonner's conclusions. The spiral may be right- or left-handed but retains throughout the same sign in any individual cell. It is a further interesting and significant feature of the wall structure that the ratio $L/b \cot \theta$, as defined in Table III, is approximately constant within the limits of experimental error. As will be seen later, this is of some importance in considerations of the growth processes in the cell, and recalls a similar conception derived in the case of some conifer tracheids. Such discrepancies as occur are capable of ready explanation. This point will be considered later; it is sufficient to point out here that the cell wall in macerated material is always somewhat wrinkled, leading to an error in the determinations of cell length and girth which it is impossible to take into account.

DISCUSSION AND CONCLUSIONS

The evidence thus obtained, both from intact cells and from single walls, is clearly in favour of a spiral organization of the primary walls of parenchyma in the oat coleoptile. There is, of course, still the possibility that the walls concerned are not uniform in cellulose chain direction—that this direction fluctuates from layer to layer much as it does in *Valonia* (Preston and Astbury 1937) and as it is claimed to do in many secondary walls. In this case no precise meaning can be attached to the direction of the major extinction position. No account was taken of this possibility in the work of Bonner, and it does seem, from considerations of wall sections, that it may be disregarded. In this view, the wall is perfectly uniform in polarized light and shows no signs of any such variability. There is, in fact, not the slightest evidence to indicate that the wall is not wound with a single molecular spiral.

Since the configuration of the cellulose component of this wall is so different from previously accepted conceptions, it clearly becomes necessary to reinvestigate the possibility of a connexion between wall structure and the development of the tissues concerned. It has been shown clearly by Tetley and Priestley (1927) that all the cells of the coleoptile are present at a very early stage and that increase in the length of this organ, during its development, is due solely to cell expansion; cell division seems to be entirely lacking in the developing parenchyma. The coleoptile presents an interesting and relatively simple case of one phase of growth, and one which can readily be attacked by comparatively simple methods. On the basis of this observation by Tetley and Priestley, an attempt has been made by Bonner, in the paper to which reference has already been made, to show that cell expansion has no effect in modifying wall structure. His interpretations are, however, invalidated since they are founded on the erroneous conclusion that the cellulose chains in the wall run transversely. Moreover, the experimental evidence presented in support of his statements is hardly adequate. This evidence consists of a series of investigations of the path difference $(n_\gamma - n_\alpha . d)$ of half coleoptiles, with the outer wall of the epidermis removed. This quantity is found to remain approximately constant for coleoptiles of widely different lengths, the dimensions of whose parenchyma are therefore presumably considerably different.* Now observations on composite material under the polarizing microscope may clearly lead to somewhat misleading results (compare e.g. Preston 1931; Preston and Astbury 1937) a point upon which considerable emphasis is to be laid (Astbury, Marwick, and Bernal 1932; Preston and Astbury 1937). In the present case it is clear that such observations may have no significance for they refer to a *tissue* rather than to a single cell; and when consideration of an intact cell leads to misinterpretations, it is sufficiently obvious that figures derived from a tissue will lead to similar consequences. No conclusions concerning wall structure in relation to growth can be acceptable, unless they are based on observation of single cell walls.

The figures already presented in Table III are sufficient to indicate the general lines along which such a correlation is to be found. The fact that the equation

$$L = Kb \cot \theta$$

is found to hold fairly accurately is of some considerable significance, particularly since a similar result has already appeared from investigation of the development of the conifer tracheid (Preston 1934), and here again can readily be interpreted in terms of the geometry of the spiral.

Consider a cell, with a spiral winding of molecular chains, cut open longitudinally and laid out flat (fig. 2*a*). Let l be the length of the spiral relating to one complete turn, L' the corresponding length of the cell, and θ the inclination of the spiral to the longitudinal axis. Then

$$L' = b \cot \theta,$$

where b represents the girth of the cell. Now if L is the length of the cell and n the number of turns of the spiral,

$$L = nL',$$

and, finally,

$$L = nb \cot \theta. \tag{1}$$

The observed correlation between the length of the cell and the cotangent of the inclination of the spiral, illustrated in Table III, thus indicates that, if the above considerations are applicable, cell expansion tends to affect wall structure in such a way that the number of turns of the spiral remains unchanged. It would certainly seem that the molecular spiral of cellulose constituting the cell wall can be treated as a series of rigid spiral threads, and that their inclination is affected by growth somewhat as this theory would predict; and that the remarks concerning this spiral made in the case of the cambial initial of the conifer (Preston 1934) are here equally applicable. Such discrepancies as occur in Table III may readily be explained in terms of (1), for change in L or b may be accommodated by change in n or θ or both. The number of turns of the spiral will, in fact, only be constant in so far as external and internal conditions will allow. It may well be that occasionally the growing cell twists so that n changes

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* In a brief communication, noticed after the writing of the present paper, Wuhrmann and Meyer (1937) show that, in etiolated coleoptiles, the path difference changes from negative at the tip to positive at the base. This would support the argument presented in the following pages. Their observations further show that the "primarsubstanz" (Hess *et al.* 1936) in the wall has no effect on the extinction positions, although the optical effects of this substance are probably more complicated than they suggest.

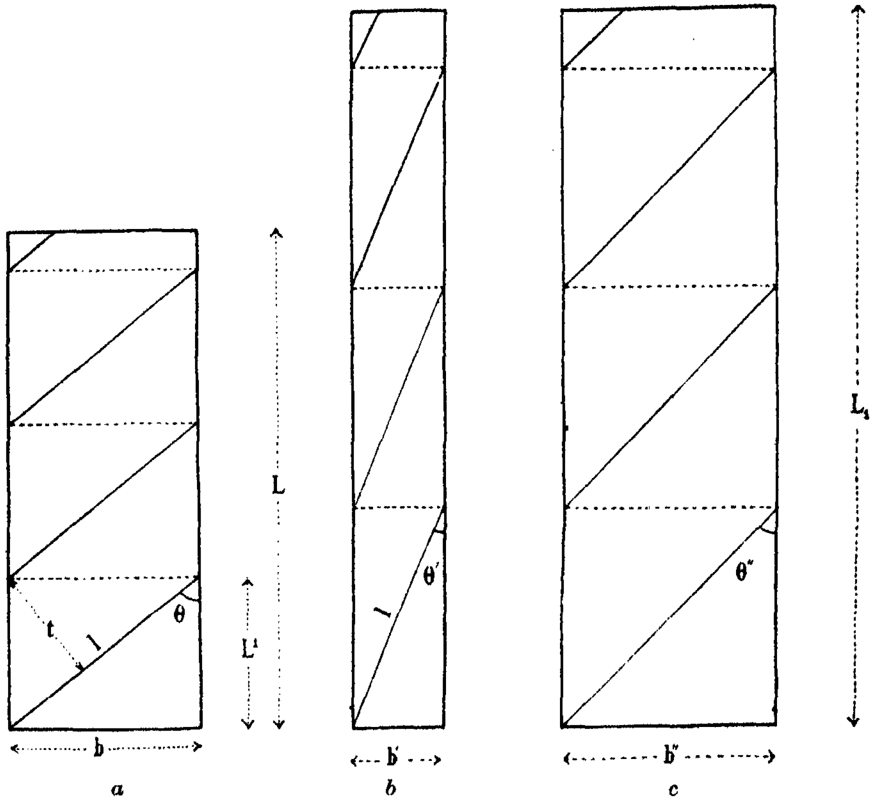

FIG. 2. (*a*) Diagrammatic representation of a cell cut open longitudinally and laid out flat. The oblique lines represent the spiral winding. (*b*) The same cell after elongation by an external, longitudinally directed tension. (*c*) The same cell after an equal elongation by growth, under the assumption that girth increases at a rate one-fifth that of length increase.

as well as θ. The data in Table III actually indicate merely a *tendency* of the cells to grow without any change in the number of turns of the spiral.

One further point demands attention. It was observed by Bonner that while a mechanical extension of only 9 % sufficed in certain cases to transform the birefringence of a half coleoptile from negative to positive, an extension by growth of 100 % failed to do so. At first sight this would seem to support his contentions. At the same time it is difficult, if not impossible, to interpret this type of observation in terms of the single cell. One point is, however, quite clear. A large part of this difference in the effects of mechanical deformation and growth is to be explained by the different geometrical features of the two cases of cell expansion. Let us consider, for instance, a cell as in fig. 2*a*. If such a cell is elongated by the application of a longitudinally directed tension so that the length L increases to L_1 (fig. 2*b*), then l will remain approximately constant and we may derive the new angle of inclination of the spiral from the equation

$$L_1 = l \cos \theta' = b' \cot \theta'.$$

On the other hand, if the cell elongates to the same length L_1 by growth (or by the mechanical forces involved in growth) then l may not remain constant (fig. 2*c*). Again, b will in general increase, or at any rate will not manifest the considerable decrease which must occur during the application of an external force. Thus, expressing L_1 in terms of b'', an increased girth, we have

$$L_1 = b'' \cot \theta'',$$

where θ'' is the inclination of the spiral after the period of growth. Hence

$$\frac{\cot \theta''}{\cot \theta'} = \frac{b'}{b''}.$$

$\cot \theta''$ is thus less than $\cot \theta'$ so that θ'' is greater than θ'. Thus the application of external forces may be expected to have a greater influence on the direction of the cellulose chains than growth alone, even though the latter has a considerable effect. In particular, if θ is only a few degrees greater than 45°, then a mechanical elongation of only a few per cent will suffice to reduce it to a value less than 45° (when the birefringence of the intact cell will change from negative to positive); whereas an extension by the forces involved in growth, producing the same effect, may be of a much higher order. Perhaps a numerical example may help to make this clear. Consider a case (Table IV) taken at random from Table III, of which the inclination is rather far removed from 45°. In view of the lack of evidence concerning the relative rates of elongation and girth increase, let us assume what would appear to be a rather low figure of 5:1. This will give us a minimal difference between the two effects. If the cell is extended mechanically by 50 %, then the inclination will decrease from 60° to 41°, and the birefringence will at the same time become positive. Extension by growth, however, will cause a decrease to only 52°, and the birefringence will remain negative. An elongation of 170 % is, in fact, required to reduce the inclination to 41° by growth alone. In the case of a single cell there is, then, a considerable difference between the elongations required, under these different conditions, to bring about a change from negative to positive birefringence. When a tissue, rather than a single cell, is to be examined, the difference may well be enhanced, since the changes observed are the resultants of the effects of many cells under widely different conditions of wall structure, and the effect of "side" walls is to be taken into account.

TABLE IV

	Dimensions			
Condition of cell	Length per turn of spiral	Length	Breadth	Inclination
Initial	0·067	0·150	0·118	60·0
After 50 % elongation by external forces	0·110	0·225	0·088	41·5
After 50 % elongation by growth	0·110	0·225	0·130	52·5
After 17 % elongation by growth	0·181	0·405	0·159	41·5

In such a geometrical treatment as the problem has thus received, the precise mechanism whereby growth processes may influence wall structure does not appear. There is, in fact, little that can be said about this point as yet. It is clear, however, that the distending forces that arise from turgor may play a role of some importance, although it is doubtful in some cases whether they are adequate to explain observed phenomena (Frey-Wyssling 1936). Whether such changes as have been described above can arise through an active growth of the wall would appear uncertain. Evidence of an indirect nature has been obtained to show that change in cell dimension, by growth of the wall, may have no effect on the direction of the cellulose particles in it (Preston 1934). Yet, theoretically, active interpolation of new wall particles might well cause precisely the same changes expected from extension by turgor forces. The distance between two consecutive windings of the same spiral thread (fig. 2a) is readily deduced to be

$$t = b \cos \theta;$$

hence as t increases (by the insertion of new particles of wall substance laterally between the old) θ may decrease if increase of b is restricted. The probability is that both turgor forces and pure growth are operating simultaneously in affecting wall structure. It is commonly observed that during growth of the primary wall little change in wall thickness occurs. This is particularly striking in the case of the cambial initials in conifers, which undergo such considerable extension (Kerr and Bailey 1934). New wall substance must undoubtedly be developing, being deposited either in the wall (intussusception in the sense of Nageli) or upon it (apposition in the sense of von Mohl, Klebs, and Wiesner). The chemical nature of the wall supports the former alternative, as suggested by Bonner and others, a point which is further emphasized by the fact that the young growing cell often fails to plasmolyse. At the same time, the fact that young walls are always plane, rather than curved or wrinkled, would suggest that they are under tension. It is therefore possible to suggest that the deposition of new wall particles is often preceded by a slight distortion of the wall, due to turgor forces, somewhat as suggested by Söding (1934; see also Boynsen Jensen 1936). This inlaying of new material causes a release of tension, the suction pressure of the cell increases, and the wall is again brought under tension. Thus the process continues; certainly not in such discrete phases as here suggested, but rather by an operation of both factors simultaneously. Extension due to growth would then appear to proceed in a "series" of deformations, which may be elastic (Pfeffer 1904) or plastic (Söding 1931, 1932, 1934; Heyn 1930, 1931, 1934) according to the rapidity with which the insertion of new wall particles follows upon extension, although its sum total is apparently plastic. The process will continue so long as turgor forces are able to cause sufficient strain in the wall; it may well be that in some cases such strain is lacking and that the crystallization forces involved in the latter of the two phases of growth play a controlling part. Clearly, in the parenchyma cells studied here, either phase can be effective in changing the direction of the cellulose chains only in so far as it affects the relative dimensions of the cell. Investigation of the effects of growth on wall structure in cells in different environmental circumstances would probably help to solve some of the problems involved in these considerations, since it is possible that a separation of the two phases might be effected.

Thus the conclusion may be reached that the parenchyma of the oat coleoptile is wound with a molecular spiral of cellulose chains, and that the inclination of the spiral changes during growth in general agreement with geometrical considerations. The inclination of the spiral changes roughly in accordance with the change in the ratio

$$\frac{\text{length of cell}}{\text{girth of cell}}.$$

If this ratio increases, then the spiral becomes steeper; if it decreases, the spiral may be expected to become flatter. It must be borne in mind that if the ratio remains constant during cell growth, then the inclination will remain constant. In special cases, therefore, apparent independence may be expected of wall structure from growth; but even such conditions arise from a balancing of opposed tendencies and would constitute no sound evidence for real independence. The precise relation between length increase and girth increase is undoubtedly controlled both by the physical properties of the wall and by external limiting factors. The relative importance of these factors will certainly differ in different cell types and we may expect considerable variation in the apparent response of the wall to growth changes.

SUMMARY

Consideration of both intact cells and of single walls in oat coleoptiles shows clearly that the cellulose chains of the wall are inclined to the transverse plane, in contradistinction to previously accepted ideas. The chains may, therefore, be represented as forming a series of spirals round the cell; and the spiral may be right- or left-handed in different cells, though it retains the same sign throughout in any individual parenchyma. Growth of the coleoptile under constant light conditions, at a temperature of 25° C., appears to involve a change in the inclination of this spiral, in accordance with changes in cell dimension, which may be interpreted in terms of the geometry of the spiral. The ratio of the length of the parenchyma to its girth is shown to be of importance in this respect. If this ratio increases, the spiral becomes steeper; if it decreases, the spiral may be expected to become flatter. It is probable that both turgor forces and active growth of the wall, as well as external conditions, play a part in this change in micellar inclination. Further investigation of coleoptiles, grown under widely different conditions, is clearly necessary before the precise value of such factors can be appreciated.

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Starch-iodine coloration as an index of differential degradation by the amylases

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Although the intimate nature of the coloured iodine compounds formed by starch and starch dextrins remains obscure, this colour reaction has played a prominent part in the study of amylases. Parallel observations on the changes in iodine coloration and the increase in reducing power during the enzymic degradation of starch led first to the recognition of the fact that amylases from different sources induce distinctive types of breakdown.

Under the action of the so-called "saccharogenic" (or β) amylase from ungerminated barley and other cereals about 60 % of the starch substance is transformed into maltose, but the iodine colour changes only to violet or mauve. In contrast, most amylases are of the so-called "dextrinogenic" type, having the capacity to destroy completely the iodine colouring property. The colour changes typically from blue, through shades of violet, rose brown, and orange, until at the "achroic point" the colour of added iodine is not altered. Enzymes from different sources are distinguishable, however, in that the increases in reducing power which accompany the destruction of the iodine colouring property vary considerably.

With a few recent exceptions (Samec 1935, 1937; Blom, Bak and Braae 1936, 1937) observations on the iodine colour have been purely qualitative. The object of the present investigation was to determine to what extent the changes in this property, when followed quantitatively, may serve to indicate the progress and type of starch breakdown by different amylases. Absorption spectra were determined at intervals during the action on starch of five representative amylases, the measurements being made on solutions containing samples of the reaction mixtures with a constant amount of added iodine. Concurrently, the liberation of reducing groups was followed by both alkaline copper and alkaline hypoiodite reagents.

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METHODS

Enzymes

The following amylases were investigated: the saccharogenic (β) amylase from ungerminated barley; the dextrinogenic (α) component from malt, obtained by the method of Ohlsson (1925); the amylase of *Aspergillus oryzae*; and the two animal amylases—from pancreas and saliva. These were stored as dry powders, prepared by the methods described in Appendix I. Only the *Aspergillus* preparation showed detectable maltose-splitting activity and this had been reduced to an almost insignificant level during the purification.

Substrate

All experiments were carried out using the same specimen of soluble starch as substrate. This was prepared by a method based on that of Small (1919) under the conditions described in Appendix II. Solutions of starch were prepared freshly for each experiment: the required weight, suspended in a little cold water, was washed into boiling water; after 3 min. boiling with stirring, the solutions were held at 100° for 20 min., then cooled and brought to volume.

The particular specimen of starch used exhibited a reducing power of 0·6 by the copper method, and about 2·5 by hypoiodite (maltose being 100). The intensity of its iodine coloration was rather higher (about 6 %) than the average of several specimens which have been examined.

Conditions of hydrolysis and experimental procedure

The final volume of the reaction mixture was normally 200 ml., the concentration of starch being 0·2 % and the reaction temperature 25° in all experiments. Each amylase was allowed to act at approximately the optimal hydrogen ion concentration. The *p*H values of the digests, and the buffers used in the different cases, were as follows: β-malt, *p*H 4·8, 0·015N acetate (final concentration); α-malt, *p*H 5·3, 0·015N acetate; *Aspergillus*, *p*H 4·7, 0·015N acetate; pancreatic and salivary, *p*H 6·8, 0·005N phosphate. With the two animal amylases 30 mg. sodium chloride per 100 ml. reaction mixture was added, this concentration having been found to produce maximum activation.

The enzyme, 2–6 ml. of solution, in a tube with vaselined lip, and the remainder of the digest in a flask, were brought separately to 25°. The reaction was initiated by pouring the enzyme into the reaction flask (zero time by stop-watch) and twice rinsing the tube with the mixture. Thereafter, samples of appropriate volume for the different determinations were withdrawn, pipettes of fairly rapid delivery being used. The first sample could be taken at about 0·6 min. after zero time.

For the observations on iodine coloration, samples of 5 ml. (equivalent to 10 mg. starch) were run into 100 ml. volumetric flasks containing 5 ml. 0·2 % iodine in 0·3 % potassium iodide. The walls of the flask were immediately washed down and the contents mixed. After making up to volume the absorption spectra of these solutions were measured. For copper reduction values, samples varying from 2–5 ml. were run directly into 5 ml. of the alkaline copper reagent. Samples of 5 or 10 ml. for hypoiodite values were delivered into 1 ml. 0·1N soda. The details of the alkaline copper and hypoiodite methods are given in Appendix III.

The enzymic action in the samples was arrested rapidly under these different conditions and no detectable changes occurred during the intervals which normally supervened before completing the determinations (0·5–1 hr. in the case of reduction values, 0·5–5 hr. in the case of absorption spectra). Control experiments showed that none of the determinations was affected by the presence of the buffer mixtures and sodium chloride in the different digests.

Measurements of light absorption

A system of approximate spectrophotometry was adopted involving the use of the step-photometer of Zeiss with a set of glass filters of relatively narrow transmission. By this means measurements of absorption could be made in nine spectral bands of which the mean wave-lengths (in A units $\times 10^2$) were 43, 47, 50, 53, 57, 61, 66, 72 and 75. The results will be expressed as extinction coefficients related to the absorption by 1 cm. of solution; in practice, the depth was varied to obtain the maximal accuracy, having regard to instrumental errors and visual sensitivity.

Sets of four to six readings were made normally in determining each extinction value. The highest accuracy was obtainable with filters 57 and 61, the individual readings agreeing to within about 1·5 % and the averaged value being reliable to within about 1 %. With filter 43 a greater experimental error was clearly related to visual fatigue and in extreme cases a scatter of 5 % was encountered in a set of readings. The values with the remaining filters were in general reproducible to within 2–3 %.*

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* It is important that the temperature of the solutions be controlled during the absorption measurements. Unpublished observations by A. M. Acock in this laboratory have shown that the extinction value for starch-iodine is influenced appreciably by temperature.

THE IODINE COLORATION OF SOLUBLE STARCH AND CERTAIN DEXTRINS

In fig. 1A are shown extinction curves for solutions containing 10 mg. iodine (with 15 mg. potassium iodide) per 100 ml. and amounts of starch varying from 1 to 15 mg. In these concentrations, the starch solutions without iodine showed no measurable absorption. The values for the iodine alone, without added starch, are plotted.

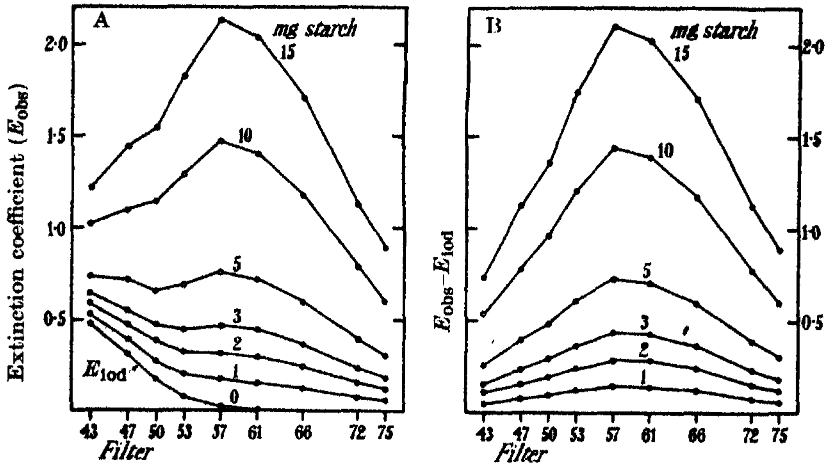

FIG. 1. Extinction curves for varying amounts of starch with constant iodine (10 mg./100 ml.). Abscissae represent mean λ of filter transmissions. A. Observed extinction values (E_{obs}). B. Derived extinction values ($E_{obs}-E_{iod}$).

With small amounts of starch it is clear that the absorption is to a large extent determined by the iodine which is present. With increasing amounts this becomes less noticeable, but it is evident that each curve represents the resultant of two sources of absorption, iodine, and the coloured iodine-starch complex.

The evaluation of these two components is facilitated by the fact that the iodine alone exhibits no measurable absorption at wave-lengths 66, 72 and 75. Accordingly, at these wave-lengths the observed extinction values (E_{obs}) correspond to absorption which is due solely to the iodine-starch compound. Over this spectral region E_{obs} is found to be approximately proportional to the concentration of starch when this is varied from 1 to about 10 mg. per 100 ml. From this it would follow that the concentration of iodine-starch compound is also proportional to the starch concentration over this range and, therefore, that the extinction values corresponding to the absorption by this compound at all wave-lengths will be approximately proportional to the starch concentration.

When the values in the shorter wave-lengths are considered, where iodine without starch exhibits absorption, it is found that the derived values $E_{obs}-E_{iod}$ (where E_{obs} is the value found for starch + iodine, and E_{iod} is the value for the iodine alone) conform with the predicted relationship of proportionality with the starch concentration.

TABLE I

	Filter								
	43	47	50	53	57	61	66	72	75
	A. Iodine alone (E_{iod})								
10 mg./100 ml.	0·480	0·317	0·180	0·079	0·029	0·010	—	—	—
	B. With starch—($E_{obs}-E_{iod}$)/mg. starch								
mg./100 ml.									
1	0·050	0·080	0·098	0·124	0·146	0·142	0·124	(0·078)	(0·060)
2	0·056	0·078	0·099	0·122	0·145	0·143	0·122	0·078	0·061
3	0·052	0·079	0·099	0·123	0·146	0·144	0·122	0·078	0·061
5	0·052	0·080	0·097	0·122	0·146	0·141	0·120	0·079	0·061
10	0·054	0·077	0·096	0·121	0·144	0·139	0·118	0·078	0·060
15	0·048	0·075	0·090	0·116	0·140	0·136	0·114	0·075	0·059
	C. With α-amylodextrin—($E_{obs}-E_{iod}$)/mg. dextrin								
mg./100 ml.									
2	0·049	0·071	0·091	0·121	0·132	0·117	0·095	(0·056)	(0·043)
5	0·050	0·073	0·092	0·120	0·131	0·114	0·090	0·054	0·040
10	0·051	0·072	0·091	0·118	0·129	0·112	0·088	0·053	0·040
15	0·048	0·070	0·088	0·117	0·127	0·112	0·089	0·052	0·039
	D. With red-staining dextrin*—($E_{obs}-E_{iod}$)/mg. dextrin								
mg./100 ml.									
4	0·034	0·046	0·042	0·039	0·029	(0·018)	—	—	—
10	0·036	0·042	0·042	0·039	0·027	0·017	(0·010)	—	—
20	0·035	0·040	0·039	0·037	0·027	0·016	0·010	(0·004)	—
30	0·032	0·038	0·040	0·036	0·027	0·016	0·009	0·004	(0·002)

* Mixed product from the action of *Aspergillus* amylase on starch.

This is shown in Table I. In sections A and B are given the values of E_{iod} and of $(E_{obs}-E_{iod})$/mg. starch for different amounts of added starch. These values for each of the filters remain approximately constant when the concentration of starch is varied from 1 to about 10 mg./100 ml. Accordingly, within the limits of error of the present method, the net increase in extinction at any spectral region provides a measure of that part of the total absorption which is due to the coloured iodine-starch compound. Curves based on these derived $(E_{obs}-E_{iod})$ values are plotted in fig. 1B. These curves, of which the form is determined by the absorption characteristics of the starch-iodine compound and the height at any wave-length is proportional to the concentration of starch, thus provide a quantitative description of the iodine colour property.

This procedure for evaluating the extinction due to the iodine-polysaccharide complex is equally valid in the case of various products of degradation. Thus in Table IC are set out the quotients (E_{obs}-E_{iod})/mg. for varying amounts of α-amylodextrin, the residual dextrin which remains after the completed action of β-malt-amylase on starch (cf. p. 404). This dextrin, it will be seen, forms an iodine complex of which the absorption characteristics show a general similarity to the iodine-starch compound, the point of maximal absorption being in the 57-filter band in each case. In Table ID corresponding data are given for the mixed dextrins obtained by arresting the action of *Aspergillus* amylase at a stage exhibiting a red-brown iodine colour. For each of these dextrins it will be seen that the (E_{obs}-E_{iod}) values for all wave-lengths are approximately proportional to the concentration of dextrin added.

It appears, therefore, that under the present conditions absorption by the iodine itself is not significantly altered by the addition of starch or dextrins but is merely masked by a new and superimposed source of absorption. It follows, either that an inappreciable fraction of the iodine is taking part in the formation of the iodine-starch compound, or that the light absorption exhibited by iodine in the free state is not appreciably altered by its participation in the complex.* Whichever alternative may be correct, the observations justify the adoption of the derived values (E_{obs}-E_{iod}) as representing, within the limits of error of the method, a measure of the extinction due to the iodine-polysaccharide complex, and accordingly a basis for following quantitatively the alteration in the iodine colouring property of starch during its degradation. For convenience, such derived values will be referred to as E values, the spectral zone being designated by the mean transmission of the particular filter, viz. E_{57}.

The effects of varying concentrations of iodine

Although the available data do not permit a full analysis of the relationship between the intensity of colour and the concentration of iodine, certain observations may be mentioned. In Table II, for example, are given

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* Preliminary experiments in which the amounts of bound and free iodine have been determined (by means of a method based on the partition of iodine between aqueous and chloroform phases) appear to favour the latter interpretation, although the evidence is not conclusive.

E_{57} values for solutions containing 10 mg. starch with amounts of iodine varying from 0·2 to 20 mg./100 ml., the concentration of potassium iodide being consistently 1·5 times that of the iodine.

With the concentration of iodine adopted as standard for the subsequent experiments, namely 10 mg./100 ml., the colour intensity is not actually maximal. Further increases in the amount of iodine, however, produce relatively small increases in colour; thus, with twice the concentration, the E_{57} value is increased by about 17 %. The adoption of a higher concentration as standard was avoided since it was desirable to keep as low as feasible the absorption due to free iodine itself, and since, with 10 mg., the intensity of the coloration was found to be approximately proportional to the amount of added starch over a suitable range.

TABLE II. EFFECT OF VARYING IODINE CONCENTRATION, WITH 10 MG. STARCH/100 ML.

Mg. iodine/100 ml.	0·2	0·5	1·0	2·0	3·0	5·0	10·0	15·0	20·0
E_{57} (measured)	0·01	0·19	0·57	0·85	1·04	1·23	1·45	1·59	1·69
E_{57} (calculated)*	(0·15)	(0·33)	0·56	0·86	1·04	1·25	1·47	1·57	1·62

* Calculated from the hyperbolic equation of best fit, neglecting the values for 0·2 and 0·5 mg. iodine.

It might be expected on the simplest hypothesis, assuming the reversible formation of a coloured iodine-starch compound by the reaction of free iodine and free starch, that the relationship between E_{57} and iodine concentration would be that of a rectangular hyperbola. While there is an approximation to this relation over a considerable part of the range there is a marked departure with low concentrations of iodine. This may be seen from Table II, in which are given E_{57} values calculated from the hyperbolic equation which gives the best fit for the values observed with iodine concentrations of 1–20 mg./100 ml. ($E_{57} = 1{\cdot}80\,[I]/[I] + 2{\cdot}21$). The further consideration of this relationship will be taken up elsewhere.

ALTERATIONS IN IODINE COLOUR DURING THE DEGRADATION OF STARCH

In fig. 2 (A–E) are plotted extinction curves determined at intervals during the action on 0·2 % starch of the five different amylases under investigation, the reaction conditions being as described above (p. 388). It will be seen that there is a marked difference in the alterations which occur under the action of β-malt-amylase, on the one hand, and the four amylases of the so-called dextrinogenic type, on the other.

From the beginning of the action of β-malt-amylase the *E* values decrease over the whole spectral range. Throughout the process, the curves display a general similarity in form to that of undegraded starch, the position of maximal absorption being at the 57-filter band.

This stands in contrast to the transformation observed during the

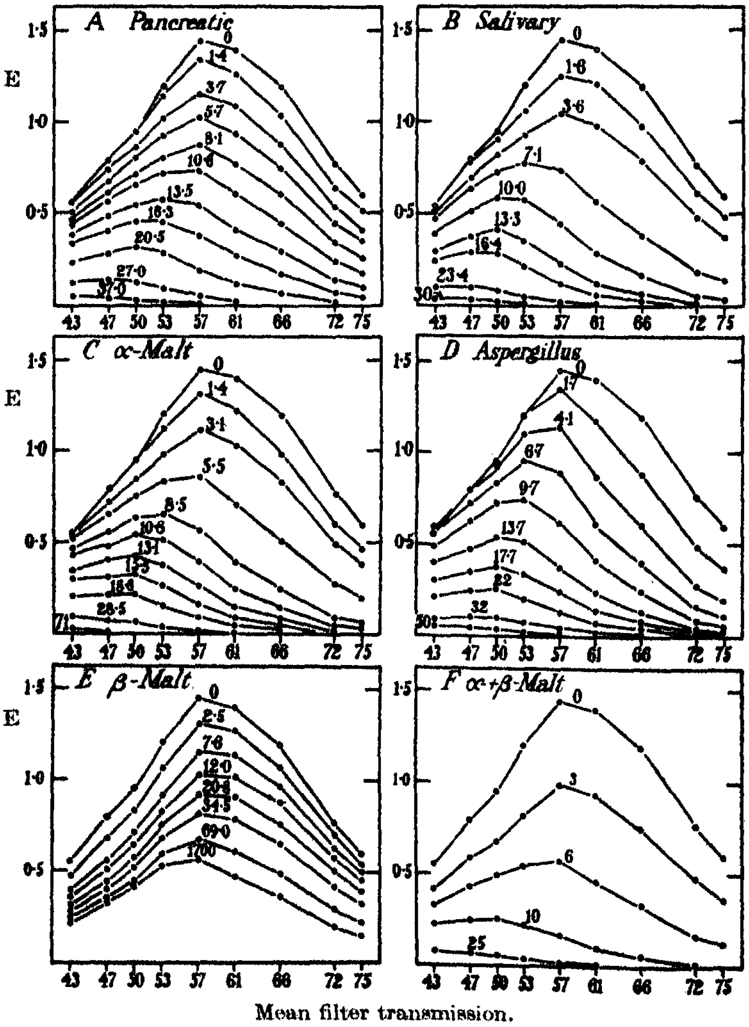

FIG. 2. Extinction curves at intervals during the action of different amylases on 0·2 % starch. The reaction time (in minutes) is indicated for each curve. A, pancreatic (0·2 mg./100 ml.); B, salivary (0·25 mg.); C, α-malt (6 mg.); D, *Aspergillus* (0·4 mg.); E, β-malt (4 mg.); F, α-malt (4 mg.) + β-malt (4 mg.).

action of α-malt, *Aspergillus*, salivary and pancreatic amylases. With these enzymes the initial stages of reaction result almost exclusively in decreased absorption of the longer wave-lengths, the values in the violet and blue remaining at first practically constant. Subsequently the values decrease over the whole spectrum but the fall continues to be more pronounced in the longer wave-lengths. Thus, as the curves become lower the position of maximum absorption shifts progressively towards the blue.

While the data for the four dextrinogenic amylases show these general features in common, the alterations are not identical. This will be seen from Table III in which, for each of these enzymes, extinction values are given at early (violet) and late (orange) stages in the sequence. At each stage the E_{57} values are the same for all the four amylases, being 1·12 at the violet stage and 0·262 at the orange.*

TABLE III. EXTINCTION VALUES AT VIOLET AND ORANGE STAGES OF IODINE COLOUR DURING DEGRADATION BY DIFFERENT AMYLASES

	Filter								
Amylase	43	47	50	53	57	61	66	72	75
					Violet stage				
α-malt	0·54	0·72	0·85	0·98	**1·12**	1·03	0·83	0·49	0·38
Aspergillus	0·59	0·78	0·92	1·10	**1·12**	0·85	0·59	0·28	0·20
Salivary	0·53	0·74	0·85	0·98	**1·12**	1·06	0·86	0·53	0·40
Pancreatic	0·54	0·72	0·84	0·99	**1·12**	1·04	0·84	0·50	0·40
					Orange stage				
α-malt	0·34	0·41	0·43	0·38	**0·26**	0·15	0·08	0·02	—
Aspergillus	0·32	0·37	0·41	0·37	**0·26**	0·16	0·09	0·03	—
Salivary	0·31	0·41	0·46	0·40	**0·26**	0·15	0·08	0·02	—
Pancreatic	0·28	0·33	0·37	0·35	**0·26**	0·17	0·10	0·03	—

It is possible merely by eye inspection to recognize slight differences in the colours observed during the action of these enzymes. An approximate idea of the relation between the measured E values and the colours of the solutions as judged visually may be gained from Table IV.

It may be stated that the results shown in fig. 2 are typical of a considerable number of observations on these amylases, and that the alterations in the E/wave-length curves appear to be independent of variations within wide limits in the enzyme concentration. It can be concluded therefore that these quantitative observations on the iodine colouring property reveal a marked difference between the saccharogenic and dextrinogenic types of amylase; further the alterations induced by the dextrinogenic enzymes, although similar, are not identical.

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* The sets of data for α-malt-amylase corresponding to violet and orange colours, respectively, are taken directly from the sequence plotted in Fig. 2 B, being the 3·1 and the 13·1 min. observations. For each of the other enzymes, the values were obtained by graphical interpolation from the sequences plotted in Fig. 2 C, D and E. This was done by plotting the E value for each filter against reaction time, and reading from these curves the values at a reaction time corresponding to the required E_{57} values.

TABLE IV. RELATION BETWEEN E_{57} VALUES AND COLOURS OF SOLUTIONS

		Dextrinogenic amylases				Saccharogenic
E_{57}	E_{57} relative	α-malt	*Aspergillus*	Pancreatic	Salivary	β-malt
1·44	Starch = 100	Blue	—	—	—	Blue
1·16	80	Brownish violet	Blue-violet	Brown-violet	Violet	Blue
0·87	60	Brown-violet	Violet	Brown-violet	Brown	Blue
0·58	40	Rose brown	Deep red	Red-brown	Rose brown	Violet
0·29	20	Deep orange	Orange	Deep orange	Rose orange	—
0·07	5	Yellow	Yellow	Yellow	Orange-yellow	—

It is clear that no single numerical value will serve as a strict characterization of the iodine coloration at any particular stage of degradation, the full extinction curve being essential for this purpose. It is convenient, however, in discussing the progress of the transformation, to make use of some arbitrary index of iodine colour. For this purpose the E_{57} value offers certain advantages; the greatest absolute change in absorption occurs at this spectral zone; the absorption by iodine alone is small, and the measurements are subject to the smallest error.

In adopting the E_{57} values as an arbitrary index of iodine coloration, however, the inherent limitation of any such numerical scale requires emphasis, that identical values do not necessarily indicate identical extinction curves.

ALTERATIONS IN IODINE COLOUR IN RELATION TO THE LIBERATION OF REDUCING GROUPS

In the experiments now to be considered parallel determinations of iodine coloration and reducing power were made during the action of the five amylases. The concentration of each enzyme was varied twofold, the amounts of the different preparations per 100 ml. reaction mixture being: β-malt, 4 and 2 mg.; α-malt, 6 and 3 mg.; *Aspergillus*, 0·4 and 0·2 mg.; salivary, 0·25 and 0·125 mg.; pancreatic, 0·4 and 0·2 mg. The substrate was 0·2 % starch throughout, the other conditions as described earlier (p. 388).

The reduction values will be expressed as percentages of the theoretical amount of maltose for complete conversion. These have been corrected by subtracting the small reducing powers exhibited by the different enzyme preparations but not the initial reducing power of the starch substrate. The values thus represent the total reducing power of the products of degradation. Frequent determinations by the copper method were made throughout the reaction with only occasional determinations by alkaline hypoiodite. The hypoiodite values, although subject to greater error, were found to be in substantial agreement with the copper values, except in the early reaction stages when they indicated consistently 2–4 % greater apparent conversion (cf. examples in Table V). This difference seems clearly to be related to the higher reducing power of the undegraded substrate toward hypoiodite as compared with copper (cf. p. 388). For the present purposes only the copper values will be considered in full.

TABLE V. COMPARISON OF VALUES BY COPPER AND HYPOIODITE METHODS

A. Pancreatic amylase (0·2 mg./100 ml.)

		Reaction time (min.)							
		6·5	11·6	17·2	21·2	36·1	95·0	203	1400
Apparent conversion	Hypoiodite	13·5	24·5	33	36	50	58	66	73
	Copper*	12·2	20·7	30·1	35·7	49·8	61·0	66·3	72·5

B. α-malt-amylase (3 mg./100 ml.)

		Reaction time (min.)							
		2·3	7·1	16·2	31·6	41·5	74·0	122	223
Apparent conversion	Hypoiodite	6·0	12·5	18·5	25	28	29·5	33	36
	Copper*	3·4	9·1	16·0	23·0	25·7	29·5	32·1	34·3

* Derived by interpolation from *R*/time curves.

The observations are shown in fig. 3A–E, in which the values of E_{57} (colour index) and *R* (reducing power) for each of the ten digests are plotted against reaction time. To facilitate a more direct comparison of the changes in the two properties, the *R* value corresponding to each E_{57} value was obtained by interpolation from the appropriate *R*/time curve. The E_{57} values were then re-calculated to a relative basis, the value of 1·44 for starch being taken as 100 (cf. Table IV, p. 396). These corresponding values of E_{57} (relative) and *R* are plotted in fig. 4. In this form the data display the "*colour/R*" relationships characterizing the different degradative processes.

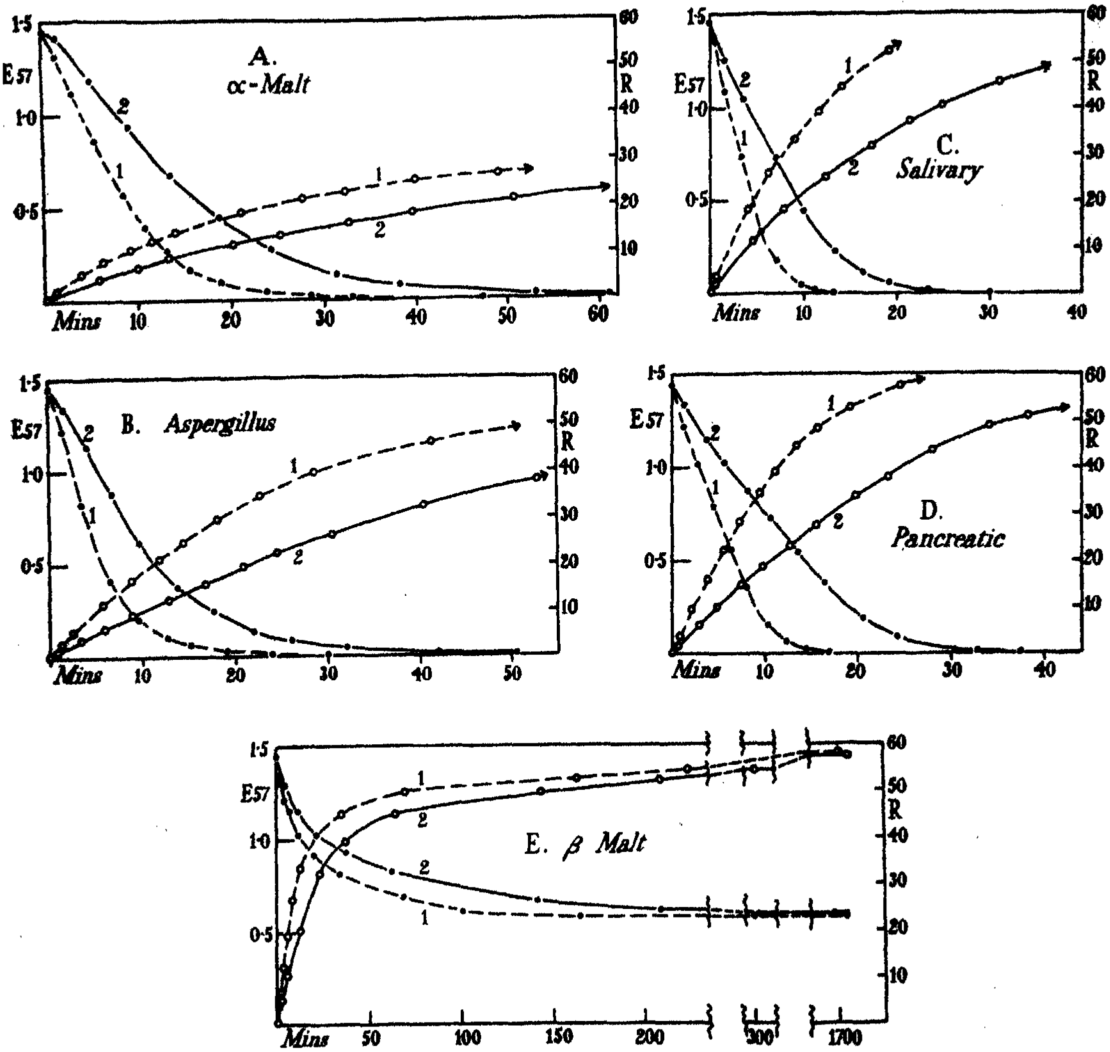

FIG. 3. Alterations in E_{57} and R values during the action of different amylases on 0·2 % starch, the concentration of each enzyme being varied twofold. E_{57} values are plotted ●; R values ○.

The points for each pair of digests by the same amylase are in close agreement, lying approximately on a single smoothed curve. Since in each case the concentration of enzyme was varied two-fold, it is clear that the *colour/R* relationship reflects a peculiarity in the *type* rather than the *rate* of the breakdown. Experiments with certain of these amylases have established the constancy of the relationship over a considerably wider range of enzyme concentrations.

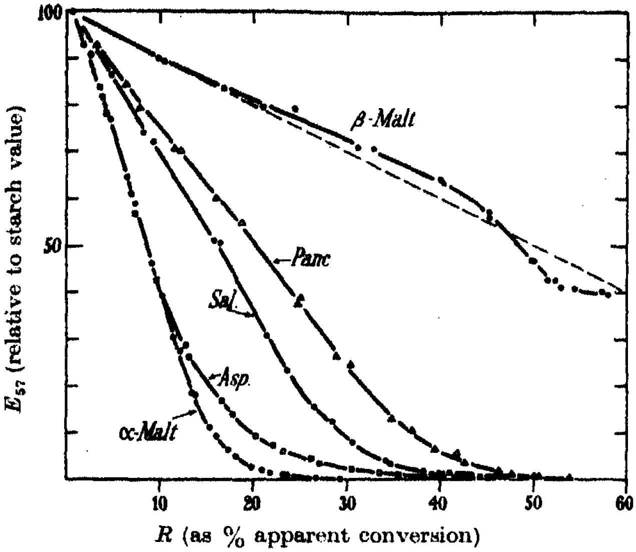

FIG. 4. E_{57}/R relationships derived from the data shown in fig. 3.

It will be seen that the four amylases of the so-called dextrinogenic type are clearly differentiated on the basis of these curves. With α-malt-, and *Aspergillus* amylases, the initial portions of the curves (to the stage at which R reaches 10 % apparent conversion, and E_{57} has fallen to 40) are similar. Thereafter the curves separate, the achroic point being attained at an R value of about 30 % with α-malt-amylase, and about 44 % with the *Aspergillus* enzyme. The curves for salivary and pancreatic amylases separate early in the reaction, and are distinguished from those for α-malt- and *Aspergillus* amylases in that corresponding decreases in E_{57} are accompanied by greater increases in reducing power. The R values at the achroic point for salivary and pancreatic amylases are about 46 and 55 % respectively.

The E_{57}/R curve for β-malt-amylase is widely separated from the curves for the dextrinogenic amylases. In this case the percentage decrease in the colour index is of the same order throughout the hydrolysis as the percentage conversion of the substrate into maltose. This may be seen by reference to the straight line which has been added in fig. 4.*

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* A further aspect of the action of β-malt-amylase may be mentioned. In both digests by this enzyme (fig. 3E) the reaction had virtually ceased after 28 hr., the

These different amylases fall into a graded series as judged by the relative rates at which the iodine colouring property is destroyed and reducing groups are liberated when they act on starch. While it has been stressed above that the selection of the E_{57} value as a numerical index of iodine coloration is purely arbitrary, it may be stated that the use of an alternative index, such as E_{43}, E_{75}, or the E value at the extinction maximum, does not alter the order in which the *colour/R* curves for these amylases arrange themselves. According to any of these criteria, α-malt-amylase forms the one extreme of the dextrinogenic group, the curve for the *Aspergillus* enzyme showing a general similarity (although not absolute identity) during the early stages. Then follow in intermediate positions the curves for salivary and pancreatic amylases, and, forming the other extreme, that for β-malt-amylase.

The observations discussed in this section are summarized in Table VI.

TABLE VI. REDUCTION VALUES AT DIFFERENT STAGES IN THE ALTERATION OF IODINE COLORATION

Colour index E_{57} (relative to starch = 100)	Reducing power (by copper) as percentage apparent conversion				
	Dextrinogenic amylases				Saccharogenic (β) amylase
	α-malt	*Aspergillus*	Salivary	Pancreatic	
80	4·1	4·1	6·7	7·6	22
60	7·1	7·1	12·8	16·0	43
40	10·0	10·0	18·8	23·9	58
20	13·4	15·0	24·9	31·8	—
0	(30)	(44)	(46)	(55)	—

THE JOINT ACTION OF THE TWO MALT AMYLASES

It has been found in experiments with mixtures of the two components of malt amylase that the E_{57}/R curves lie in positions intermediate between the curves which characterize the separate action of these enzymes. With

R values corresponding to about 59 % conversion. Similar values for the "limit of hydrolysis" have been sometimes observed (cf. Baker 1902; Hanes 1935; Blom and others 1936). Depending on the source, and the preparation of the enzyme, a further extremely slow hydrolysis may be observed beyond this state, the R value rising to 67–70 % apparent conversion. This secondary slow conversion, which has also been reported by other workers (e.g. Baker 1902; Ling and Nanji 1923), we find to be accompanied by a disproportionately large decrease in the iodine colour. The detailed discussion of this secondary process, which we are not inclined to attribute to β-malt-amylase itself, will be undertaken in a separate paper.

appropriate proportions of the two, E_{57}/R relationships coinciding approximately with those found for salivary and pancreatic amylases may be observed. In such cases, however, the duality of the enzyme becomes evident when the full extinction curves are examined. A typical example is shown in fig. 2F. The transformation shows a blending of the effects observed during the separate action of the two enzymes. Thus, the extinction values in the short wave-lengths decreases from the beginning of the reaction, as occurs with β-malt-amylase alone, while the preponderating fall in the values at the longer wave-lengths is characteristic of the dextrinogenic amylases.

On the basis of the E_{57}/R relationship alone, it might have been considered possible to reproduce by mixtures of α- and β-malt-amylases a system similar to pancreatic amylase. This illustrates, however, the limitation of any simple numerical colour index (cf. p. 396), since the full E/wave-length curves suggest strongly that, whether or not the pancreatic amylase consists of more than one enzyme, it is unlikely that a component similar in its action to β-malt-amylase is present.

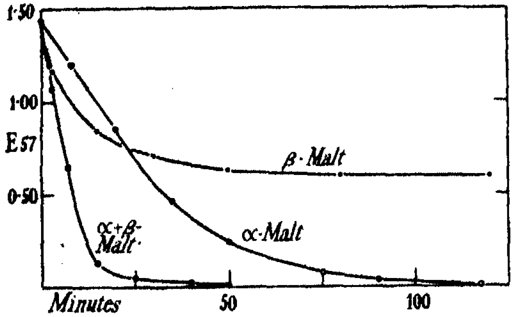

FIG. 5. Alterations in iodine colour during the separate and joint action of β-malt (5 mg.) and α-malt (1·5 mg.) on 0·2 % starch.

One fact established in such experiments deserves particular emphasis. Numerous observations have shown that, although the action of β-malt-amylase by itself does not result in the complete destruction of the iodine colouring property, yet the presence of this enzyme together with α-malt-amylase hastens markedly the attainment of the "achroic" condition. In fig. 5, for example, the alterations in the E_{57} values during the action of β-amylase (5 mg./100 ml.); α-malt-amylase (1·5 mg./100 ml.) and these same amounts of the two enzymes added together (starch concentration 0·2 %; pH 5·0). In the presence of β-amylase the time required for the disappearance of the iodine colour was reduced from 140 to 55 min.

It is clear, therefore, that with mixtures of the two malt amylases, such as occur naturally in germinated barley, the rate of destruction of the iodine colouring property is by no means an exclusive function of the amount of the α-component. This fact is of considerable practical importance since the time required for the attainment of the achroic stage is often regarded as yielding a specific measure of the concentration or activity of the α-component in malt amylase, an interpretation which is clearly erroneous.

THE BEARING OF THESE OBSERVATIONS ON THE PROBLEM OF THE CONSTITUTIONAL BASIS OF THE IODINE COLOURING PROPERTY

In spite of the recent important advances toward the elucidation of the molecular constitution of starch, little light has been shed on the nature of the iodine compounds formed so characteristically by starch and the related glycogens. This colour reaction is usually held to be due to the adsorption of iodine related to the colloidal state of these substances. It is safe to assume, however, that the uptake of iodine depends ultimately upon the presence of residual affinities of a specific character. The particular atomic group or groups responsible for such affinities remains to be established.*

According to the Haworth-Hirst conception, which has been reviewed in detail elsewhere (Hanes 1937*a*), the chemical unit of starch is considered to consist of a chain of 24–30 α-glucopyranose units linked in positions 1 and 4 as in maltose. Observations on a number of starch dextrins have demonstrated that these represent fragments of the chain structure containing varying numbers of glucose units. It seems clearly established, however, that in starch itself, and certain types of dextrin, the chemical units do not exist as free individuals, but that these are associated together to form molecular aggregates. The natures and position of the linkages involved in such associations remains obscure.

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* Jackson and Hudson (1937) have prepared, by the action of periodic acid, an oxidized derivative of starch which is devoid of iodine coloration in spite of being highly colloidal in nature. In this product the individual pyranose rings are opened as a result of the formation of aldehydic groups in positions 2 and 3, but little fragmentation of the chains themselves appears to have occurred. These observations suggest that the colouring reaction depends upon residual affinities associated with the pyranose rings of the chain structure. The observations of Bergman (1924) on certain cyclo-acetals which form blue-black iodine compounds suggested that the oxygen bridge is an integral part of the "colouring group". The fact that fully-methylated starch derivatives retain the property (indicating that the hydroxyl groups are not involved) lends interest to this view.

The iodine colours exhibited by starch products of which the chain lengths have been determined are of considerable interest. The shortest fragment so far reported to give a colour with iodine is a dextrin fraction with a mean chain length of about 8 glucose units. This product, and in addition a dextrin of about 12 units (both of the disaggregated and non-retrograding dextrin type), were reported by Haworth, Hirst and Plant (1935) to give red colours with iodine. From other observations it appears that fragments of chain lengths up to about 6 units do not colour with iodine, although the molecular dimensions are not so definitely established —cf. the apparently low-molecular "maltodextrins" described by early workers, and the dextrin of about 6 units described by Waldschmidt-Leitz and Reichel (1934). There is thus a suggestion that a minimal length of chain of about 7–8 glucose units may be essential for the coloration.

It is clear, however, that the actual hue of coloration is determined mainly by some factor other than the chain lengths as revealed by the chemical method. Thus, the starch of waxy maize, which gives a red-violet colour with iodine, is not distinguishable as to the length of its chemical unit from starch varieties which exhibit the normal iodine colour (Haworth, Hirst, and Woolgar 1935). A corresponding example is afforded by the red-staining non-aggregated dextrin of 12 units, mentioned above, and the violet-staining α-amylo-dextrin for which a similar value (11–12 units) was reported by Haworth, Hirst, Kitchen and Peat (1937). α-Amylo-dextrin, in contrast to the former product, exists in an aggregated state, and undergoes retrogradation in solution.

It appears therefore that chain molecules containing approximately the same number of glucose units may exhibit appreciably different colours with iodine. This must be attributed either to the existence of constitutional differences actually within the chain molecules themselves, or to differences in the condition of these chemical units in the different products (cf. Samec and Waldschmidt-Leitz 1931). If the first view were adopted, the existence of two (or more) distinct species of "colouring group", yielding iodine compounds of different hue, might be postulated; differences in the colours exhibited by products of the same chain length might then be explained as arising from the presence of varying proportions of these groups within the molecular chains of the different products. According to the second interpretation, some factor affecting the chemical units as a whole, such as the mode of their association, or possibly the degree of hydration, would be held to determine the hue of the coloration. Two hypotheses might then be advanced in explanation: (1) the colouring groups might be considered to be uniform in character, the absorption properties of the iodine compounds being altered in some way by the organization of the chains into molecular associations; or (2) it might be postulated that two or more species of colouring group are present, of which one type at least is peculiar to molecular associations. According to either of these conceptions, the breakdown of molecular associations would be accompanied by a transformation in the iodine colouring property.

While the available chemical evidence does not exclude definitely any one of these interpretations, the current view that the units and linkages throughout the chain structure are of an identical type would favour either of the latter two conceptions. In discussing the results of the present experiments, however, the three possibilities will be considered equally.

Degradation by the saccharogenic (β) amylase

In the case of β-malt-amylase there is strong support for the view advanced elsewhere (Hanes 1937*b*) that the degradative process consists in the cleavage of successive terminal disaccharide fragments from one end of the chain molecule (probably the non-aldehydic end). At each stage there is present, in addition to maltose, a dextrin molecule of starch-like properties which represents an intact residual portion of the original structure. The process continues normally until 58–60 % of the starch substance is transformed into maltose, at which point some obstruction to the further action of the enzyme is encountered and there remains a resistant fragment of the molecule which constitutes α-amylodextrin.

According to this view the chain length (and the amount) of the "amyloid" dextrin residue would diminish in inverse proportion to the extent of the conversion of the substrate into maltose. On the basis of the value of 24–30 glucose units for the chain length of starch, a homogeneous degradation of the chains to the stage of 60 % conversion would yield a residual molecule 10–12 units in length, a prediction confirmed by the observations of Haworth, Hirst, Kitchen and Peat (1937) on α-amylodextrin. The yield of α-amylodextrin, moreover, is approximately 40 % (Hanes 1935; Haworth and others 1937).

An important aspect of the process is that the cleavage of maltose is not accompanied by the complete disruption of the associated state of the molecular residues. Thus, the entire series of amyloid dextrins retain the characteristics of the aggregated state.* Evidence will be considered elsewhere which indicates that these residual products correspond to the portion of the starch molecule which includes the potentially aldehydic end-group, and that degradation by β-malt-amylase proceeds from the opposite end. This suggests that it is the portion of the molecule toward the aldehydic end which is concerned mainly in the process of molecular association.

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* Various grades of aggregation, involving possibly distinct mechanisms of association, are clearly recognizable in starch products. The transformation of native starch into soluble starch, for example, must be attributed to a profound alteration

In the light of this conception of the mode of attack by β-malt-amylase, the alterations in the iodine colouring property during the process are of interest. The broad features established are that the extinction curves retain a resemblance in form to that of starch itself, but that the intensity of the coloration decreases approximately in inverse proportion to the extent of the degradation. Since at any stage the concentration of amyloid dextrin may be derived from the maltose value, and since this forms the sole product exhibiting an iodine colour, it is possible, from the observed extinction values and the corresponding R values, to calculate values of E per mg. amyloid dextrin at different stages of degradation. Such values (derived from observations shown in fig. 2a) are given in Table VII.

TABLE VII. IODINE COLORATION OF AMYLOID DEXTRINS. EXTINCTION PER MG. DEXTRIN CALCULATED AT DIFFERENT STAGES OF DEGRADATION

Stage of degradation of starch	Filter								
	43	47	50	53	57	61	66	72	75
0	0·054	0·078	0·096	0·122	0·145	0·140	0·122	0·078	0·061
9·3	0·052	0·075	0·092	0·117	0·144	0·140	0·118	0·077	0·059
24·9	0·0525	0·0745	0·095	0·112	0·153	0·151	0·127	0·0835	0·066
32·7	0·053	0·0755	0·0955	0·123	0·152	0·150	0·130	0·085	0·067
44·9	0·0525	0·076	0·096	0·123	0·147	0·142	0·118	0·076	0·059
51·3	0·051	0·073	0·093	0·122	0·139	0·125	0·100	0·061	0·046
56·0	0·050	0·077	0·097	0·120	0·129	0·110	0·087	0·048	0·036

It will be seen that the iodine colorations developed by the amyloid dextrins up to the stage of about 45 % conversion are strikingly similar to that of starch itself, the colour intensity with certain of these products

in the state of aggregation yet even in the case of soluble starch there is little doubt that the chemical units still exist as molecular associations. Similarly in the action of β-malt-amylase on starch paste a marked decrease in viscosity occurs, which is not explicable solely on the basis of the reduction in length of the chains. Since the residual dextrins retain the properties of aggregated products (e.g. insolubility in cold water after drying, strong opalescence in solution, the capacity to retrograde in solution) it is clear that the decrease in viscosity in this case is the result mainly of a disruption of certain higher states of aggregation, leaving intact molecular associations of a more intimate nature.

being actually slightly higher than with starch. Beyond the stage of about 50 % conversion a falling off in the absorption of the longer wave-lengths occurs but there is still a general similarity to the starch values. Data for an isolated specimen of α-amylodextrin (at the stage of 59 % conversion) have already been given in Table I, p. 391.*

In spite of the considerable range in the chain lengths of this series of products (from 24–30 to 10–12) it is clear that they exhibit iodine colorations which are not markedly different from the original substrate. It must be concluded, therefore, that the end-wise attack by this enzyme which results in a progressive shortening of the chains is not accompanied by any considerable alteration in that factor which determines the hue of the iodine compounds. The aggregated condition of the residual products suggests that the associated condition of the molecular chains is important in this connexion.

The observations on β-malt-amylase would thus be explicable on the assumption that iodine colouring groups of at least approximate uniformity occur at fairly regular intervals along the molecular chain structure. The progressive cleavage of maltose fragments from the chain end would then be pictured as resulting in the destruction of successive iodine colouring groups so that at any stage in the process the number remaining would be roughly proportional to the concentration of the residual dextrin. If, alternatively, the existence of two or more types of groups forming iodine compounds of different colour were postulated, it would be necessary to assume that these are homogeneously distributed along the chains, so that the different species of groups would be destroyed in approximately constant proportions during the degradation of the structure from one end. A consideration of the observations on the dextrinogenic amylases appears to render untenable this interpretation.

Degradation by the dextrinogenic amylases

It is not yet possible to formulate precisely the manner in which the starch molecule is degraded under the action of any amylase of the so-called dextrinogenic type. It appears that there first occurs a cleavage into complex dextrins of reducing character, these being broken down in turn to yield shorter fragments, including maltose (cf. Köhler-Hollander

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* Although small changes in the iodine colouring property appear to occur when these products are precipitated and dried, direct observations on the amyloid dextrins isolated at different stages of the degradation of starch have confirmed the essential features shown by the calculated values in Table VII. The products isolated at 23 and 40 % conversions were found to yield significantly more intense iodine colours than undegraded starch when examined in identical concentrations.

1934; Freeman and Hopkins 1936). This process is accompanied by a progressive alteration and ultimate destruction of the iodine colouring property.

While it has been found that the action of four examples of this amylase group results in approximately similar changes in the iodine colour, it is clear that the degradative processes are different in these cases. This is evident not only from differences in the *colour/R* relationships but also from peculiarities in the course of the reaction in the presence of these different amylases. Thus, in fig. 6A are shown typical progress curves (*R/time*) for α-malt, *Aspergillus*, salivary, and pancreatic amylases. The concentrations of enzyme were such as to result in approximately similar initial rates of hydrolysis in each case, but to facilitate further a comparison, the time scales have been adjusted to produce a superposition of the tangents to the different curves at zero time.

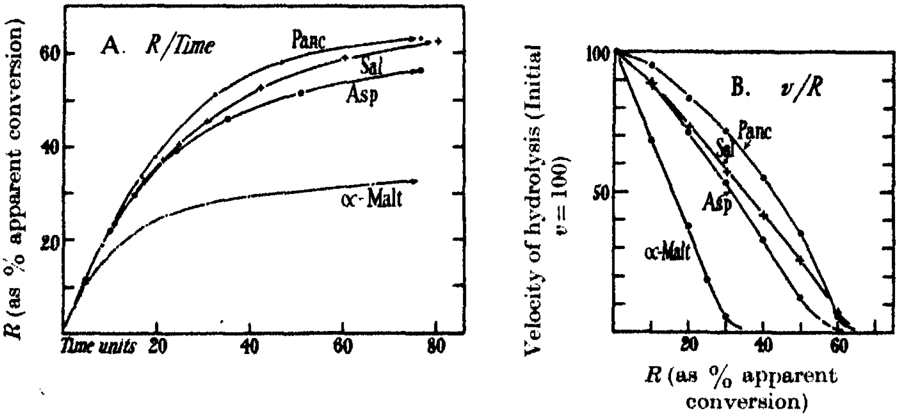

FIG. 6. The course of the reaction with the four dextrinogenic amylases. A. *R*/time curves. Time scales: 1 unit is equal to 1·175 min. for pancreatic, 1·00 min. for salivary, 1·17 min. for *Aspergillus*, and 1·47 min. for α-malt. B. Velocity/*R* relationships derived from the curves shown in A.

With α-malt-amylase, the velocity falls off early and after the stage of 25–30 % apparent conversion, hydrolysis proceeds extremely slowly. This curve stands in marked contrast to that with pancreatic amylase, in which hydrolysis proceeds smoothly to over 60 % apparent conversion, a marked decrease in velocity becoming noticeable only at the stage of about 45–50%. The curves for salivary and *Aspergillus* amylases fall between these two extremes, the latter exhibiting a slightly more pronounced falling-off in velocity as the reaction proceeds. These features are more clearly shown in fig. 6 B in which the reaction velocity (determined from the slopes of tangents to the curves at appropriate points) is plotted against the stage of degradation (the R value). It will be seen that the four amylases fall in the sequence α-malt, *Aspergillus*, salivary and pancreatic, as judged by these v/R curves, and further that this is the same order as when they are arranged on the basis of the *colour*/R curves (cf. fig. 4). Thus with α-malt-amylase, which forms the one extreme, the velocity falls off earliest in the reaction and at the same time a given alteration in the iodine colour is accompanied by the smallest number of hydrolytic cleavages; on the other extreme, with pancreatic amylase a high velocity is maintained to the most advanced stage, and the transformation of the iodine colour is accompanied by the cleavage of the largest number of linkages.

A relatively simple hypothesis which would account for the broad parallelism between these two aspects of the different processes of breakdown may be advanced. It might be assumed that achroic dextrin fragments are liberated in a primary cleavage under the action of each of these enzymes, and that these are further hydrolysed in a secondary process. The relative rates of these two reactions, which would be pictured as proceeding concurrently, would vary if the different enzymes were characterized by different capacities to attack the two postulated classes of substrate, starch and complex iodine colouring portions of the molecule, on the one hand, and the simpler achroic dextrin, on the other. Accordingly, on the assumption that α-malt-amylase exhibits the lowest affinity for the simpler substrate, as compared with its affinity for starch, and conversely, that pancreatic amylase exhibits the highest, the other enzymes being intermediate in this respect, the differences both in the general forms of the progress curves and in the *colour*/R relationships would be explicable. According to this hypothesis the so-called dextrinogenic amylases would be considered to be generically related, the differences in their action being conceived as quantitative rather than qualitative in nature.

While final conclusions as to the relationship between these degradative processes must await a closer characterization of the products than has hitherto been achieved, the feature shown in common by these amylases deserves consideration, that is the alteration in form of the extinction curve of the iodine compounds during their action on starch. An examination of the early part of the reaction reveals most clearly this differential alteration in the absorption at different wave-lengths. Thus, with each of the four dextrinogenic amylases, an initial reaction phase is observed during which the E_{43} value remains virtually constant, whereas at medium and long wave-lengths, the values fall from the beginning (cf. fig. 2 A–D).

This initial alteration in the iodine colouring is accompanied by the liberation of extremely few reducing groups. In a typical example shown in fig. 7 (5 mg. α-malt-amylase per 100 ml.) the E_{43} value begins to decrease when the reducing power reaches 7–8 % apparent conversion, by which stage E_{57} and E_{75} have already fallen to 60 and 30 %, respectively, of the original values. An examination of all available sets of data for the four amylases has shown that approximately similar values (ranging from 5–8 % apparent conversion by copper, and 7–10 % by hypoiodite) mark the stage at which E_{43} begins to fall. These values correspond on a stoichiometric basis to one reducing group per 20–35 glucose units, that is, to the liberation of not more than about one aldehydic group on an average in each of the chain molecules revealed by the chemical methods. The characteristics of this initial transformation would appear to render untenable the conception that the molecular chains contain *per se* different species of iodine colouring groups and that the observed alteration is the result of the differential destruction of these groups. Such an interpretation could require the assumption that about 70 % of the groups responsible for absorption in the 75-filter band are destroyed, while the number absorbing in the 43-band remains unchanged. It appears unlikely that this could result from a single cleavage within the chain molecule, particularly in view of the evidence derived from the β-malt-amylase that, if dissimilar groups occur, these must have a roughly parallel distribution along the chain.

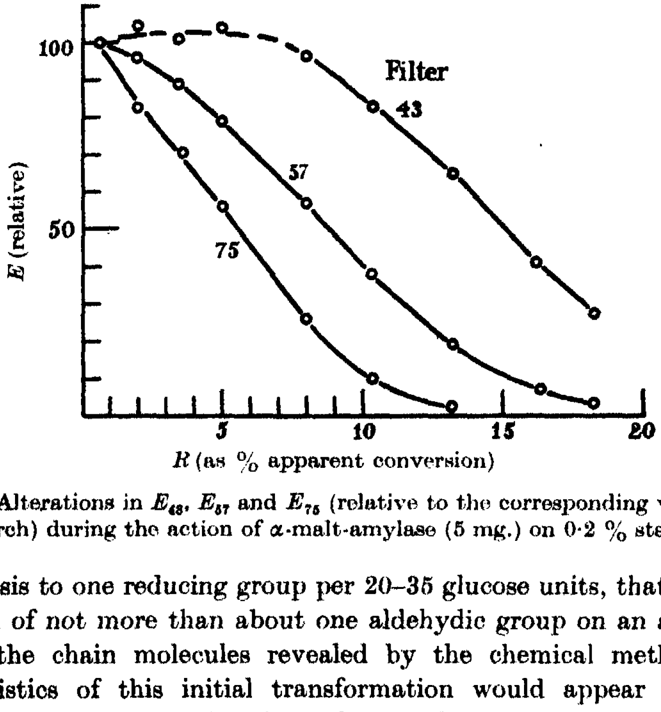

FIG. 7. Alterations in E_{43}, E_{57} and E_{75} (relative to the corresponding values for starch) during the action of α-malt-amylase (5 mg.) on 0·2 % starch.

The alternative interpretation that the altered iodine coloration is the result mainly of a change in some factor such as molecular association finds support, moreover, in the marked alteration in the physical properties of starch which occurs during the early stages of action of dextrinogenic amylases. Thus the viscosity falls rapidly, and the products, after precipitation in alcohol and drying, dissolve readily in cold water, yielding clear solutions which exhibit little retrogradation (cf. Freeman and Hopkins 1936). These facts suggest that a breakdown of molecular associations occurs early in the process. It becomes accordingly a matter of considerable interest to determine the position of the reducing group which is first liberated under the action of dextrinogenic amylases, particularly since according to one hypothesis (cf. Haworth, Hirst and Isherwood 1937) the possibility is considered that the terminal aldehydic unit of the starch chain may be engaged in the formation of molecular aggregates, thus accounting for its low reducing power. In this case disaggregation might be accompanied by the liberation of the terminal groups in a reducing form.

ACKNOWLEDGEMENTS

We wish to record that certain preliminary observations, in which measurements of iodine coloration were made with the Lovibond Tintometer, were carried out by one of us (M. C.) at the Botany Department, Oxford, in 1934. These experiments, which were suggested by Dr W. O. James, formed the starting point of the present investigation. We are grateful to Dr F. F. Blackman, F.R.S., for criticism of the manuscript.

SUMMARY

The alterations in iodine coloration have been followed by quantitative measurements during the action of different amylases on starch. The so-called saccharogenic and dextrinogenic types of amylase are shown to be distinguishable, almost from the beginning of their action, by the manner in which the absorption characteristics of the iodine compounds are altered. Further, it becomes possible to delineate accurately, throughout the course of each degradative process, the relation between the changes in the iodine colouring property and the liberation of reducing groups. The observations on five representative amylases indicate that these full *colour*/R relationships (which have been presented arbitrarily as E_{67}/R curves) form a diagnostic criterion for comparing the mode of action of different members of this group of enzymes.

While the observations clearly afford a basis for considerable refinement in methods available for investigating the action of amylase, these applications will be discussed elsewhere. It has seemed preferable to consider the bearing of the data on the problem of the constitutional basis of the iodine colouring property of starch products.

The observations can be accommodated on either of two hypotheses. According to the first, a single species of "colouring group" would be considered to occur at regular intervals along the chain structure, and the absorption characteristics of the compound formed by the attachment of iodine at this group would depend upon the mode or degree of association of the molecular chains of a particular product. Alternatively, two (or more) distinct species of "colouring group", yielding compounds of different hue, might be assumed to be distributed in parallel along the chains, but of these one species, at least, would be assumed to exist exclusively in molecular associations. It will be seen that these admissible hypotheses both incorporate the conceptions (1) that the "colouring groups" are distributed homogeneously along the molecular chain structure such that different portions of the molecule are approximately equivalent in this respect, and (2) that differences in the hue of iodine colours exhibited by different products are related mainly to the phenomenon of molecular association. (A third conception that the glucopyranose chains *per se* contain distinct types of colouring group appears untenable.)

Adopting either hypothesis, the observed alterations in iodine colour during the action of β-malt-amylase are conceived as resulting from a progressive reduction in the number of "colouring groups" during the cleavage of successive maltose fragments from the end of the chain. The fact that the intimate molecular associations of the residual dextrins are not broken down during this process would account for the approximately constant absorption characteristics of the iodine compounds. Conversely, in the case of the dextrinogenic amylases, the marked alteration in the hue of the iodine coloration is attributed mainly to a preliminary disruption of molecular associations, this being followed by a progressive destruction of "colouring groups" as the chains are cleaved into achroic dextrins.

APPENDIX I: PREPARATIONS OF ENZYMES

Saccharogenic (β) amylase (from barley)

100 g. flour from ungerminated barley (Canadian, O.A.C. 21 variety) was stirred for 2·5 hr. with 375 ml. 50 % alcohol. The suspension was centrifuged and filtered. To 250 ml. filtrate was added 1·2 g. calcium

acetate. After standing for 2 days at 1°, a heavy precipitate was removed and discarded. Alcohol to 83 % was added; the resulting precipitate was suspended in 50 ml. water. On adding alcohol to 50 % a slight precipitate formed; this was removed and discarded and alcohol to 80 % was then added. The precipitated enzyme was washed exhaustively in alcohol (95 %, then absolute) and ether, and dried *in vacuo*. In contrast to earlier products (cf. Hanes 1935) this preparation dissolves readily in water yielding a clear solution. At *p*H 4·6 and 25° it liberated four times its own weight of maltose per minute from 0·5 % starch.

Dextrinogenic (α) *amylase* (*from malt*)

800 g. flour from germinated barley (dried at 30°) was stirred for 5 hr. with 1500 ml. water. After centrifuging and filtering, alcohol to 60 % was added to the filtrate. The precipitate was suspended in 500 ml. water, and this was heated rapidly to 70°, held for 15 min. at this temperature, and cooled rapidly. After centrifuging and filtering, alcohol to 60 % was added to the filtrate. The resulting precipitate was then washed in alcohol, and ether and dried.

Amylase of Aspergillus oryzae

This enzyme was prepared from a commercial sample of taka-diastase (Parke, Davis and Co., undiluted taka-diastase powder) by applying the adsorption method which Holmbergh (1933) developed for the purification of α malt-amylase.

3 g. of the commercial preparation was dissolved in 100 ml. water and 100 ml. 95 % alcohol was added; a resulting precipitate was discarded. The alcoholic solution was cooled to 1° and 25 g. rice starch, previously washed in 50 % alcohol, was added. After 10 hr., the starch was removed on the centrifuge, and washed twice with 60 % alcohol. The enzyme was now eluted by washing twice in 100 ml. water with 10 min. stirring. The combined aqueous extracts were centrifuged. The enzyme was then precipitated by adding 1200 ml. 95 % alcohol, 250 ml. ether, and 0·1 g. sodium acetate; it was washed in alcohol and dried.

As a result of this procedure the activity/dry weight ratio was increased about eight-fold, and the maltase activity was reduced to a low level.

Pancreatic amylase

3 g. dried pancreas powder (pig) was dissolved in 100 ml. water. To this was added 105 ml. 95 % alcohol and 0·7 g. calcium acetate. The copious precipitate which resulted was removed and discarded. The enzyme

was now precipitated by adding alcohol to 87 %. It was washed in alcohol and dried.

Salivary amylase

To 370 ml. of filtered human saliva was added 200 ml. 95 % alcohol and 1·0 g. calcium acetate. The resulting precipitate was discarded. Alcohol to 80 % was added to the filtrate; the precipitate was washed in alcohol, and ether, and dried.

APPENDIX II: PREPARATION OF SOLUBLE STARCH

A suspension of 400 g. potato starch (air dry) in 1 l. of 95 % alcohol is brought to the boil; 10 ml. concentrated HCl is added through the condenser, and boiling, with vigorous stirring, is continued for 12 min. The product is then washed exhaustively in distilled water. It is then washed successively with 95 % alcohol, absolute alcohol, and ether. After the removal of organic solvents at 40° *in vacuo*, the moisture content is determined by drying small samples at 110° in high vacuum in a vessel communicating with cold P_2O_5.

The reducing power of different specimens of soluble starch prepared in this way have been found to vary between 0·5–0·8 by the copper method and 2·5–3·0 by hypoiodite (maltose = 100). The iodine colour developed by different specimens varied in intensity within a range of about 7 % but the absorption curves do not differ significantly in form.

APPENDIX III: DETERMINATIONS OF REDUCING POWER

(*a*) *Alkaline copper method*

A copper solution based on Reagent 60 of Shaffer and Somogyi (1933) was used; in the present modification, however, potassium iodide was added only after the completion of the oxidation, and the reagent itself was stored in two parts: A, 15·0 g. $CuSO_4.5H_2O$/l.; B, 80 g. anhydrous sodium carbonate, 50 g. Rochelle salt, and 1·50 g. potassium iodate/l. Equal volumes of solutions A and B were combined freshly for each day's work.

The determinations were made with 5 ml. of this reagent in boiling tubes (6 × 1 in.) to which were added the digestion samples, water being added, where necessary, to bring the total volume to 10 ml. The tubes were covered and heated by immersion in boiling water for 15 min. The remaining operations were carried out as described by Shaffer and Somogyi (*loc. cit.*).

Since the reduction values will be expressed in terms of maltose equivalents, the values observed with anhydrous maltose are given:

Maltose, mg.	0·3	0·5	1·0	1·4	1·8	2·2	3·0
N/100 thiosulphate, ml.	0·51	0·85	1·75	2·50	3·26	4·03	5·56

(*b*) *Alkaline hypoiodite method*

Samples of reducing power equivalent to 3–8 mg. maltose were run into 1 ml. 0·1N sodium hydroxide. 3 ml. 0·1N iodine was added, followed by the dropwise addition of 4·5 ml. 0·1N soda occupying 2–2·5 min. After 15 min. at room temperature, the mixture was acidified with 1 ml. 20 % sulphuric acid, and the excess iodine titrated with N/30 thiosulphate.

Under these conditions the iodine consumption by pure maltose was 1–3 % greater than that calculated for the stoichiometric oxidation of the aldehydic groups.

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A discussion
on
The application of quantitative methods to certain problems in psychology

24 *March* 1938

Opening address

By Dr C. S. MYERS, F.R.S.

Whatever be the conscious experience which is being quantitatively studied, the psychologist realizes that it can never be measured numerically in its own terms, but only in terms of the antecedent physical stimulus, the concomitant neural and other histological activities, or the consequent changes in outward behaviour. We can never say that one experienced sensation of sound has twice the intensity of another, or that one person has twice the experienced courage of another.

It is now generally admitted that the mathematician who concerns himself with the ultimate data of physics is not primarily interested in the physical significance of the variables with which he deals, and that his conclusions are not necessarily verifiable by experiment nor acceptable in practice by the experimental physicist. So too in psychology: if mathematical operations on the data of psychological experiment are to be of value to the psychologist, each step in such operations must be carefully studied so as to be assured that it is psychologically warranted.

We have to assure ourselves that the applications of recent developments of statistical method to psychology are consonant with the view that the psychical whole is more than the sum of its parts, that those parts are immeasurably greater than the few which we are taking into account, that not only is the nature of the whole determined by its parts but that also the nature of the parts is dependent on the whole. We have, in a word, to consider the justifiability of psychological atomism and the limitations of psychological mechanics.

Mathematical methods are *essential* in psychology when the investigation concerns the conduct of an aggregate of individuals, as in the problem of industrial accidents; but when it concerns the individual, statistics are rather *ancillary*—as a safeguard against unreliable results. In neither case can the psychologist afford to neglect them.

Correlations in psychology are dependent on measurements of objective responses solely. Factorial analysis mathematically yields 'factors' the psychological nature of which can be interpreted indirectly only and with a variable degree of uncertainty. The necessity of accepting these mathematically deduced factors as psychologically real demands consideration: what is mathematically true, or what is a possible mathematical interpretation, may have no psychological (or physical) existence. Consideration needs also to be given to the reverse statement which has been sometimes made by psychologists, that a conclusion may have psychological, although it has no statistical, significance.

Even if any single 'factor', for example, general intelligence or mechanical ability, has a real psychological existence, its elementary or complex nature, and, if complex, the possible variations of its component parts, need to be considered.

Moreover, the utility of the conception and determination of factors has to be considered in relation to the practice of occupational guidance and selection. Can one ever hope to base success in any one occupation on the possession of certain limiting amounts of different factors? And even if this possession were ascertainable, would the use of that knowledge be, on psychological grounds, justifiable and sufficient? It may turn out that factorial analysis, although mathematically feasible, is of limited value in applied practice. Nevertheless, even with its present, less 'scientific', and admittedly imperfect methods, occupational guidance and selection are demonstrably of considerable practical value.

Professor C. E. SPEARMAN, F.R.S. *New methods of expressing ability in factors*

It is hard to agree with the preceding speaker's challenge to mathematical psychology in general. Even if we concede to him that one sensation cannot be twice as intense as another, at least it can be twice as *frequent*; and that fact affords just as much scope for mathematical handling.

Very different from any such distrust of mathematics in general is the curiosity which may be felt towards the particular mathematical theory concerned with 'factorization'. In this, an attempt is made to express the seemingly chaotic multitude of abilities or other traits in a very small number of underlying 'factors', 'elements', or 'components'.

Up to a very recent date this endeavour was confined to what has been named the theory of Two Factors. It began by observing that the intercorrelations of any set of abilities tend, under certain conditions, to fall into a 'hierarchical' arrangement. And from this observed arrangement was inferred, mathematically, that each of the abilities could be expressed as the sum of two factors, the one 'general' and the other 'specific'. By means of many such simple inferences, combined and organized with psychological considerations, it was found possible to build up a general theory of mental ability.

Within the last year or two, however, some much more ambitious methods have been brought forward. One associated especially with the names of Hotelling and Kelley (1935) consists in regarding the measurements of the traits as constituting an ellipsoid of points determined by Cartesian co-ordinates. The axes of these co-ordinates are then rotated so as to lie on the principal axes of the ellipsoid. In this position they are taken to be the required factors or 'components'. Here the factorization is no longer, as in the original method, built up gradually by successive stages intimately dependent on various psychological considerations. Instead, it comes into being as a complete general solution of the problem, regardless of the psychological or any other circumstances of particular cases.

But, unfortunately, this emancipation from all particular conditions appears to have been bought at the price of introducing inacceptable general ones; indeed, such as are arbitrary and even irrational.

The second of the fundamentally new methods, that which is entitled 'centroid' and comes from Thurstone (1935*a*, *b*), has many points of resemblance. For it, too, depends on the use and rotation of Cartesian co-ordinates, although very differently applied.

Again, like the foregoing method, it proffers a general solution of the whole factorization, regardless of the particular circumstances. Naturally enough, then, this method, no less than the other one, tends to introduce artificialities and irrationalities. But this time these defects are frankly acknowledged. They are destined for removal by a second rotation superposed on the first one. This supplementary step, however, appears to bring in some formidable new difficulties, especially those connected with the usage of *oblique axes*.

There remains yet a third fundamentally new method to be considered here. It is called 'bi-factor' and has been quite lately contributed by Holzinger (1937). Like both the already mentioned methods, it accomplishes its work in a single step. But unlike them it does not claim to supply any general solution. Instead, it is especially fashioned to give information and verification on certain points; those which previous investigations have shown to be of predominant importance. By this new orientation of Holzinger's method, all the cardinal defects noted in the other two would seem to be avoided.

Professor G. H. THOMSON. Opinions regarding mental factors depend largely upon whether we are dominated by the theoretical or the practical aspect. On the practical side factorists have hoped to improve vocational and educational advice. But, mathematically at least, this is not possible, for using factors merely means using new co-ordinate axes, and cannot add to the efficiency of prediction without factors (Thomson 1936*a*). Moreover, since they outnumber the tests, the factors themselves have to be estimated. In addition it is usual to throw away the specific factor variance, although this may be correlated with the occupation. The circumstances under which it is known that this is not so are precisely those in which a direct estimate without factors can easily be made. There appears to be a direct conflict of principle between factor methods, which confine themselves to reproducing the *correlations*, and methods which endeavour to use all the information.

The Thurstone (1935*a*) system *maximizes* the variances of these disregarded specifics (Thomson 1938*a*). The proposal of M. S. Bartlett thereafter to estimate the common factors by minimizing the specifics (Bartlett 1935, 1937, 1938; Thomson 1938*b*) is therefore of peculiar interest, but involves assigning to each candidate definite degrees of excellence in qualities uncorrelated with those measured.

On the theoretical side the factor theory has been a spur to many investigators. Thurstone's conception of 'simple structure' among factors (Thurstone 1935*a*) is a striking example of the feeling that mathematical elegance must indicate underlying reality. It is to be feared, however, that factors defined by different Thurstone batteries may be incompatible, and that test loadings will not remain invariant from one battery to another. At some stage it seems necessary to have the factorial composition of a test defined by a psychologist, and thereafter *held* invariant. Criteria to ascertain when 'simple structure' can be attained have recently been developed (Thomson 1936*b*; Ledermann 1936).

The analysis of tests into factors is very dependent on the sample of persons tested. Selection can create and destroy factors and change their relations (Thomson 1937, 1938*c*), and this makes factors appear as fluid descriptive mathematical coefficients rather than realities of any sort. The influence of selection is particularly important in vocational work, where selected groups are necessarily dealt with.

The tendency among psychologists to be impressed by the small number of common factors required to reproduce correlations appears to be unjustifiable. This tendency to low rank is a mathematical necessity if the causal background is structureless (Thomson 1935). Its presence in mental measurement is an indication, not that the mind is composed of separate factors, but that it is comparatively undifferentiated and plastic.

Professor C. BURT. *Recent developments of statistical method*

The previous contributions have dealt with developments in statistical method within psychology; my own deals with those outside the psychological field, which psychologists have largely ignored.

The most important are the methods of testing significance in small samples. Believing that statistical methods are valid only for large numbers, recent investigators have abandoned the individual studies to collect less accurate data by group tests. Instead of concentrating on analysis of correlations, the various methods of analysing variance and covariance might usefully be applied, whenever the standard deviation is not arbitrary.

The methods of factor analysis now appear, like those of multiple correlation, to be simply a fresh application of the old principle of least squares. Once this is realized, many further extensions seem possible: for example, when we are endeavouring to discriminate between two or more groups—the two sexes, two antithetical temperamental or vocational types—we find that the factor that accounts for the greatest amount of the variance proves also to be the factor that discriminates most widely between the individuals or groups (cf. Fisher's 'discriminant functions').

Perhaps the most fruitful points of contact between the mathematical problems of the modern psychologist and those of other scientists are problems for which matrix algebra or tensor notation has been recently adopted. If Spearman's factor theorems, or his formula for the correlations between sums and averages, be proved afresh by matrix algebra, the student is astonished, not only at the simplicity of the proof, but at the way it can at once be generalized to as many factors as we like. The analysis of a set of test scores to find principal factors is analogous to the determination of a spectral set of projective operators by a canonical reduction. When one of the regular methods is followed, the variances and the saturation coefficients of Thurstone emerge as the first approximations, and those of Hotelling and Kelley as the final values, for eigen values and eigen vectors respectively. This appears to be the only method of factor analysis which will lead back to the same saturation values as those from which the correlation matrix was originally constructed. The analogy at once suggests many further developments (e.g. if the factors of the psychologist are not to be relevant solely to the particular table he is studying at the moment, then it must be possible to reduce two or more of such matrices to canonical form simultaneously: a modification of a familiar proposition in quantum-theory states that such a reduction is possible if, and only if, their product is symmetrical).

It is often objected that the sensory qualities and sensory intensities with which the psychologist deals are not, strictly speaking, magnitudes that obey the laws of addition and multiplication: they do, however, conform to the requirements of group theory. The 'simple percept', like the simple atom, turns out to be a pattern or structure—a structure that has to be described without knowing either the material of which it is composed or the operations which it endures. The aim of the early experimentalist was to connect an isolated sensation with an isolated stimulus of magnitude R, by some simple bivariate law such as $S=K \log R$; the problem of the contemporary psychologist is to account for the fact that, in spite of ceaseless changes in position, perspective, illumination, and the like, I always recognize my chair as my chair, and that in a single glance. It may, I suggest, be solved by regarding both percepts and objects as matrices, and the permanent external object that we invoke to explain the 'phenomenal constancy' as a matrix reduced to standard form.

Dr H. B. HEYWOOD. In factorial analysis psychologists have erected a mathematical structure with little basic help from mathematicians in that correct understanding of 'factors' on which its soundness depends.*

Factors are a degenerate form of orthogonal functions. A sequence of scores $x_1, x_2, \ldots, x_n$, may be regarded as a point function of the variable $1, 2, \ldots, n$, and can consequently be developed in series of orthogonal (or other) functions. However, to reach the notion of factors, which has grown out of that of partial correlation, we must measure the elements x from their mean, so that their sum becomes zero, and normalize them so that the sum of their squares is unity. Factors are sets of orthogonal functions, subject to these same two conditions, in terms of which the sequence x may be expressed linearly. Given any number of sequences of scores, it is possible to express them all linearly in terms of the same set of factors in a widely infinite number of ways. The same may be said of sequences of meaningless numbers.

The case of psychology is very different from that of physics. In physics the orthogonal functions appropriate to a given problem are uniquely determined by body equations, continuity and boundary conditions. In psychology these equations and conditions do not exist, and consequently the factors for a given population are indeterminate. The Spearman hypothesis, the origin of the method of factorial analysis and of the conception that factors might be 'faculties', was based on an observed tendency in scores. In cases where it is applicable it reduces the degrees of freedom of choice to one. Godfrey Thomson and others have extended the ideas of Spearman to more general arrays. These resolutions which have considerable mathematical interest, depend upon the selection of tests and scales of measurement.

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* I have tried to contribute to this understanding in a previous paper (Heywood 1931).

Before factorial analysis can yield us a theory of intelligence we have still to discover laws founded on observation which will define a unique set of factors. Even if this were done the unique set would apply only to a single population, and when transferred to others the factors would lose their zero-sum and orthogonal properties. In the absence of an ideal or universal population there would appear to be no good reason for retaining these properties. It might be better to seek functions of general application. Were these found or devised, we should still be faced with the difficulty of admitting that human intelligence could be resolved into components, like a uniform physical entity.

Though the method may fail to produce an acceptable theory of intelligence, it may yet prove to be a useful way of analysing and codifying data of a statistical nature, and in any case there will remain the vast amount of valuable observation and empirical results which it has inspired.

Dr J. O. IRWIN. It seems frequently to happen that psychologists think a result has been proved by statistical method while the statistician, seeing that it follows correctly from the data, thinks that some observational truth of psychology has been discovered. The danger lies in the borderland where the transition from psychology to statistical method takes place. Errors in mathematical reasoning can usually be detected by competent mathematicians, but the accidental introduction of wrong assumptions or postulates at the beginning may be a much more illusive business and there may be room for much difference of opinion among psychologists as to what are the right assumptions.

Often the assumptions seem glossed over with an extraordinary naïvety in the effort to get numerical results. Thurstone's linear scale of criminality seems to be an example of this. It is at least arguable that the differences between types of crime are not quantitative at all, or if they are quantitative they are not unidimensional. By forcing the results on to a linear scale we may be vitiating the answer to the problem we are interested in. It is for psychologists to say whether this is so or not, but a much more critical attitude of mind to such questions, than seems customary, is highly desirable.

A return to the viewpoint of the Grammar of Science would bring us a much clearer idea of the place of mathematical and statistical method in psychology. According to that view scientific method consisted in the formation of conceptual schemes which are a sort of shorthand résumé of the facts of experience. The function of mathematics or statistics was to find the graduation which fits the facts. The modern statistical viewpoint is essentially similar. We speak of hypotheses instead of graduations and regard it as the function of mathematics and statistics to work out the consequences of these hypotheses, then to test them and see whether they are in accordance with the facts of experience. This has the merit of emphasizing that of two formulae which fit the facts equally well that will be preferable which is more relevant to the problem under investigation. For example more than one particular factor set-up may be consistent with the observed facts; which set-up is to be preferred is then a matter of its psychological relevance, essentially a problem for psychologists to decide.

Dr Myers has said that the mathematician who concerns himself with the ultimate data of physics is not primarily interested in the physical significance of the variables with which he deals, and that his conclusions are not necessarily verifiable by experiment nor acceptable in practice by the experimental physicist. I do not know whether this is so or not, but statisticians when they have worked out and tested the consequences of particular hypotheses and found them inadequate to describe the data of observation say that they are wrong, however mathematically interesting, and must be rejected.

When a statistician says that a result is not significant he means that it might, with a stipulated minimum degree of probability, have arisen by chance. If this is so he says the effect under examination is not proven. He has no objection to the effect being regarded as an indication which might be worth following up in further experiments, and in this sense psychologically significant. At most he might issue a *caveat* against undertaking an expensive scheme of experimentation on very slender evidence of a real effect.

Dr W. STEPHENSON. Factor analysis is still too abstract and purely statistical, and insufficiently related to psychological needs. There is required a practical factor science, which, alike for its close connexions with psychological hypotheses and its adequate representation in factor theorems, would be more like Spearman analysis than any other. In Spearman work, analysis is in terms of 'reference values', so bringing a certain continuity to the many matrices examined.

The purely analytical, fractional, nature of factor analysis has been criticized. But there is also possible a synthesis, factors being built up to serve social or industrial or other practical ends. Moreover, for many purposes we do not need to contemplate purely additive, fractional, factors.

Statistical methods can be applied to the individual as an individual. Little has been done in this direction as yet, but much work is under way. Q-technique (for persons as variables) can be applied to only *one* person if need be, when the object is to acquire information pertinent to general rather than to individual psychology. The importance of the technique in general experimental psychology will soon become manifest.

Professor Burt has put forward the claims of co-variance analysis, instead of correlational. Unfortunately, variance for most of the variables we are concerned with in psychology is a purely arbitrary matter. We can make the variance of any mental test, for example, roughly whatever we care to make it. Any analysis of such arbitrary constructions, therefore, can be of little value to psychology, if the analysis depends primarily on the variances. Correlational work is therefore inevitably the basis of most work in factor psychology, because it does not concern variance, which depends for its size upon the mere whims of the psychologist.

Statistical applications in relation to accidents and industrial problems

Professor M. GREENWOOD, F.R.S. The work to which I shall briefly refer is of limited scope but relevant to the general subject of this discussion.

In 1918, Mr Udny Yule and I happened to see some returns from factories of the numbers of employees who in a given period of time had sustained 0, 1, 2, 3, etc., trivial accidents. The distributions interested us as statisticians and we were led to ask ourselves what distributions would be expected were (*a*) all the persons exposed to risk equally liable to sustain accidents, (*b*) personal liability to vary from individual to individual.

We showed (Greenwood and Yule 1920) that, if (*a*) were true, the distribution should be that usually known as a simple Poisson series, a law of distribution which, as Professor R. A. Fisher has shown, has a wide range of usefulness in both theoretical and practical statistical work. We also found that if the law of distribution of individual liability or proneness, as it came to be called, were the limiting form of a skew binomial, then the accident distribution should be a simple series the parameters of which were easily calculated from the mean and variance of the data.

A preliminary survey of data (Greenwood and Woods 1919) from munitions factories suggested that the latter form did describe fairly well distributions of accidents. This impression was confirmed on wider data by the late Dr Ethel Newbold (Newbold 1926) who, in 1927, broadened our arithmetical basis and provided exact quantitative criteria (Newbold 1927). Since then much field work has been done both in testing the hypothesis on ampler data and giving quantitative precision to the concept of 'proneness'.

Mr Eric Farmer and his colleagues have done pioneer work on the data of industrial accidents; Dr May Smith on the aetiology of industrial sickness. They will describe their own investigations. I wish simply to emphasize the fact that such value as the work of Mr Yule and I may have had has been in calling attention to the possibility of *measuring* a factor the importance of which had been dimly recognized yet practically ignored. The saying that some people have no fingers but are all thumbs must be of immemorial antiquity, but, so far as I know, few people had asked why some people were 'all thumbs' and fewer still had sought an arithmetical measure of the disability. Our arithmetical criterion had the merit of simplicity; a very simple test (see Newbold, 1926, pp. 25–7), within the competence of an office boy, showed whether it were worth applying in any particular case. It has been found to have pragmatic value. Without doubt more accomplished mathematical statisticians, in the light of further research, will subsume it under a more general and equally manageable 'law', but it at least served the purpose of attracting attention to quantitative problems of great theoretical and even greater practical importance.

Dr MAY SMITH. *Sickness absence in industry*

The attempt to substitute measurement for vague impressions has helped to define some important problems concerning industrial sickness.

We are dogmatically assured at intervals that this or that disease is ruining the health of the community, that such and such an occupation is detrimental to health, that on the one hand medical and welfare departments in an organization merely lead to increased absenteeism, or on the other hand that they are in every way an advantage. Similarly any change may be defended or advocated on the grounds that it has increased or will increase output, or that it has decreased or will decrease sickness, when the evidence for the statements may be a comparison between two vague impressions, or in some cases between a vague impression and an accurate measurement. Actually there is very little evidence by means of which we can estimate the amount of sickness in particular organizations and occupations, or its distribution and kind.

A systematic survey extending over a period of 10 years has enabled a beginning to be made for clerical work and light industries where the conditions are good. Naturally, at first, dependence had to be placed on records kept by firms for their own purposes, and it was hoped that by a detailed study of a few organizations it might be possible to find out what was a reasonable amount of sickness absenteeism under given circumstances.

The first problem was to find a measure, and the most useful proved to be the average number of days lost through sickness per year per man or woman. Applying this measure to a number of organizations where the conditions were reasonably comparable, the range proved to be surprisingly wide, namely, from about 3 days to 11 days per man, and from 5 days to 18 days per woman.

Analysis showed that, keeping age and general conditions constant, women had a higher absenteeism rate than men.

When other conditions were comparable the factory worker had a lower rate than the clerical worker, and the clerical worker than the shop assistant.

Organizations where there was either contractual or virtual security tended to have a higher rate than those where the labour was more mobile; this however was offset in some organizations by a good initial selection and by favourable general conditions, such as recognized adequate chances of promotion for efficient work, the temperamental as well as the technical suitability of the people in authority, and stability but not rigidity of organization.

In every group the greatest loss was due to colds and influenza, accounting roughly for between 30 and 40 % of the total sickness loss.

A study of prolonged sickness absence showed that a relatively small proportion of the staff accounted for a relatively large proportion of such absence.

Dr M. Culpin. An investigation into telegraphists' cramp in 1925 (Smith, Culpin and Farmer) opened up the study of psychoneurotic conditions in industry, a field in which there was no previous experience. There was found a preponderance of nervous symptoms in the cramp subjects, as well as an unexpected prevalence among the non-cramp.

Was the nature of the employment such as to stimulate the production of symptoms, or was there a tendency for nervous people to select themselves for the employment? Learners at telegraphy were next examined and classified into three groups: those with no symptoms, those with slight and those with severe symptoms. The numbers for each group, including both sexes, were 54, 27 and 19, and these proportions are in accord with later experience, the learners showing no signs of selection in favour of, or against, those with nervous symptoms. The symptoms elicited were chiefly irrational fears or exaggerated diffidence.

The records up to 1936 are now available. Of 17 boys placed in the 'serious symptom' group 16 remain, one having been transferred to another department. In every case the supervising reports may be regarded as satisfactory.

Of the 16 boys included in the 'slight symptom' group 11 remain in the service, one having resigned, three having been transferred to other departments, and one not traced. Of the 11 remaining, 9 have satisfactory reports.

Of the 22 boys in the 'no symptom' group, only 14 remain, of whom 9 are satisfactory.

Even in the absence of established standards, for 94 % of a group of adolescents to be successful in their vocation after a ten years test is highly satisfactory.

This is at first sight a paradoxical result, for it indicates that the nervous group have settled down to their work more successfully than the rest. But the original suggestion of a tendency for nervous subjects to select themselves for this kind of occupation is confirmed, the selection taking place during the period of adjustment to a secure but routine employment.

On account of loss through marriage, conclusions relevant to this enquiry cannot be drawn from the figures on the female side.

One may look at these results from the other way round and deduce that non-nervous adolescents possess qualities which lead them to remove themselves from that particular kind of occupation represented by telegraphy. If a number of adolescents were similarly classified at their entry into, let us suppose, the Air Service, the results might be expected to turn out quite differently.

E. FARMER. *Quantitative methods employed in the study of accident proneness*

The work that Chambers and I (1926, 1929, 1933, 1936) have done in trying to verify the hypothesis of accident proneness on psychological grounds is based on the statistical work that Professor Greenwood has described. Assuming the validity of the statistical methods by which the hypothesis of unequal initial liability to accidents was established, we have tried to measure some of the individual characteristics differentiating those with a high accident rate from their more fortunate companions exposed to equal risk.

The tests we have used have covered a wide field of mental activity, ranging from tests of intelligence to the simplest sensori-motor tests. We have, however, concentrated our efforts in a particular field and the only positive results in which we feel much confidence at present have been yielded by a set of sensori-motor tests constructed by Dr Edgar Schuster. We have called these 'aesthetokinetic tests' to differentiate them from sensori-motor tests in general. The intercorrelations of these tests, yielded from twenty-four groups of subjects and comprising approximately 4000 individuals, had a mean value of 0·24, and they showed considerable consistency from group to group. They correlate with intelligence tests to the order of 0·15 and with tests of mechanical aptitude to the order of 0·19.

From the available data we draw the conclusion that these tests have a common factor but that this factor is in low saturation, so that specific factors play a large part in their performance. These tests differ in form but in each the subject is required to respond by rapid and accurate co-ordinated movements to different afferent stimuli.

The relation between these tests and accidents is similar in magnitude to that between the tests themselves, and the results have shown a fair consistency. It seems, therefore, that these particular tests measure that aspect of the accident situation which is due to poor sensori-motor co-ordination and that a closer relation between the tests and accidents is not to be expected than the one which exists between the tests themselves, on account of the large part played by the specific factors in all forms of sensori-motor co-ordination.

It is not possible to demonstrate the relation between these aesthetokinetic tests and accidents in groups of subjects with a wide age range, because sensori-motor co-ordination deteriorates rapidly with age. The accident rate also drops with age, so that these two tendencies run contrary to one another in groups with a large age range and mask any existing relation between performance in the aesthetokinetic tests and accidents. In addition the test and accident relationship cannot be demonstrated in groups with a small range of accidents. When the individuals in the group are not sufficiently differentiated by their accident rate, sampling errors tend to mask the relation between test performance and accidents. Even in groups with a wide accident range and a low age range the relation between test performance and accidents is not very close, so that sampling errors play a more important part in masking it than would be the case if the relation were more marked

Theoretical considerations, and also the correlations between these aesthetokinetic tests and accidents, make it clear that only one aspect of accident proneness has been measured by these tests. The results obtained, however, are sufficient to show that it is possible by quantitative psychological methods to predict the future accident rate of individuals exposed to equal risk. Such predictions will never be accurate in regard to particular individuals, but they may attain a reasonable accuracy in regard to types of individuals. This may, therefore, be a practical means of reducing accidents. It has been shown that those who fail in our present tests in the skilled occupations in which our subjects were engaged, have an accident rate approximately two and a half times as great over a five-year period as those who passed and were exposed to equal risks.

The aesthetokinetic tests have also been applied to groups of motor-bus drivers and have yielded positive results, although the relation between test performance and motor accidents is not so close as it is in the case of industrial accidents.

We are now making a more intensive attempt to see if a relation can be measured between accidents and some of the higher mental functions and certain aspects of emotional life, but the work is not yet sufficiently advanced to report progress.

The attempt to measure accident proneness by quantitative methods has been beset by peculiar difficulties. It has been necessary to employ the usual statistical methods of analysis with great caution, since the nature of the frequency distribution of accidents differs considerably from the normal material of the statistician. In spite of this, however, it has been possible to indicate a relation between psychological tests and accidents in certain spheres of industrial life. The relation, though small, manifests itself consistently and may be taken as indicating a step forward in methods of reducing accidents.

Sir FREDERICK MARQUIS. Having received in early life the training of a scientist and later training in the hard school of business, I must be expected to approach this subject from the angle of one who has been trained in both criticism and precision.

Scientists should not discuss psychological tests for 'Industry' as though industry represented some concrete quantity for which one could test. Different forms of industry require diverse qualities contrasting, for example, creative ability and capacity for detail; mental and physical agility; quickness of mind and soundness of judgment. Complementary with such qualities are those of temperament that affect personal relations. All these represent qualities called for by different phases of industry, and before we start talking about 'Industrial Selection', we first have to determine what makes up industrial capacity in all the different aspects of industry.

The psychologist should start by analysing the successful employer so as to determine what causes the employer to select certain people for certain sorts of jobs. The organization that I control is engaged in running department stores and selling goods, and I have been trying to determine some of the factors in selection of staff that make for success. Junior staff at the age of 16 have to be selected either for selling or office work, and thus there are two points at which a psychological test would be advisable:

(*a*) At entrance—to determine suitability for engagement.

(*b*) At the age of 16—to determine suitability either for office work or for selling.

In my organization of Lewis's, Ltd., every candidate below the age of 18 has for several years been subjected to an entrance test. The original test, prepared with expert psychological help, did not help on question (*b*), and so the single test was replaced by two groups of tests; the first to measure general intelligence and the second directed towards those qualities thought to be necessary in a saleswoman. The results were followed up, and while no correlation was observed between success in the first part of the test and success for selling, there was a positive relation as compared to success in the second test.

My problem is to determine whether or not a candidate for employment at the age of 14 will have developed into a capable saleswoman at the age of 18. This must necessarily depend to some extent on the external influences acting during this period, so responsibility for these results must not be attributed solely to psychological test!

Difficulties are raised by the choice of quantitative methods to be used in determining success in selling. Sales cannot be taken as the sole criterion, and departmental managers vary in their methods of forming judgement. The reaction of the individual to the manager will often affect results seriously.

Two qualities that no intelligence test can really assess, play a large part in success in retail selling—personality and temperament.

I do not want to leave any impression that I do not believe in psychological tests. In Lewis's, Liverpool, we are at present engaged in revising our tests in the hope that we may improve them still further. During the four years in which we have had our present special aptitude test in force, the quality of our staff has improved, and it may well be that the tests have made their contribution to this improvement. If psychological research can produce a reliable and valid test to determine at 14 who are going to be good saleswomen at 18, I shall not be slow in adopting it.

Wing-Commander R. H. STANBRIDGE. An investigation into the occupational selection of aircraft apprentices of the Royal Air Force has recently been carried out in which psychological tests were combined with an interview and a medical examination to enable data to be obtained under such headings as physique, temperament, character, intelligence, mechanical aptitude and mechanical ability.

From this, assessments were made under the following headings:

A. Those likely to do well, including potential cadets.
B. Those unlikely to do well, including those considered unsuitable.
C. Those needing further observation physically.
D. Those who should have been given a different trade.

On completion of the three years course, they were examined in schools and workshops and given passing-out ranks, as follows:

(1) Cadets and Leading Aircraftmen.
(2) Aircrafthands, First Class.
(3) Aircrafthands, Second Class—including discharges.

The assessments given soon after entry (by myself) were then correlated (by the Industrial Health Research Board of the Medical Research Council) with passing-out rank (given by the appropriate authorities).

Description of quantitative tests. No attempt is made to describe the whole investigation in detail and reference will only be made to those tests which were measured and marked on a definite scale, giving a normal frequency curve when performance marks were plotted, so making correlation possible with passing-out rank.

(1) *Psychological type.* Kretschmer classification using Wertheimer measurements and formula.

(2) *Dynamometer.* An index figure produced by dividing result of three pulls by body weight.

(3) *40 mm. mercury test.* Sustaining by blowing a column of mercury at 40 mm. pressure, noting total time and pulse rate in five second intervals.

(4) *Mechanical aptitude.* Thirteen printed mechanical problems.

(5) *Mechanical ability.* Two practical tests specially devised, based upon requirements in two different R.A.F. workshops.

(6) *McDougall Schuster dotting machine.*

(7) *Intelligence test.* The standard Group 33 test of the National Institute of Psychology.

(8) *Perseveration test.* Based on Pinards' work.

Results. 1100 boys have been investigated and records analysed statistically.

It was found that those noted to do well contained three times as many Cadets and Leading Aircraftmen as would be expected by chance, and contained no Aircrafthands Second Class or Discharges.

That those noted as unlikely to do well, contained no Cadets or Leading Aircraftmen, but over twice as many Aircraftmen Second Class and Discharges as would be expected by chance.

Of the particular tests referred to above, the Intelligence, Mechanical Aptitude and Mechanical Ability tests were found to be the most valuable and statistically significant and, combined with the normal entrance examination, gave a closer relationship with trade proficiency than did the entrance examination alone. This combined method should pick out a set of boys with an increased number in the higher ranks and a decreased number of failures and unsatisfactory apprentices.

No significant relationship was found between the 40 mm. mercury test, the psychological type, or the dotting test and the passing out rank. The perseveration test alone failed to yield useful results, although a character assessment in which it was included, showed a slight significance.

A significant interrelationship between tests was shown in the case of intelligence, mechanical aptitude, mechanical ability and dotting.

Conclusion. With reference to Dr Myers' opening remarks, if one is entitled to draw any conclusion at all from the investigation I have outlined, it would appear that some of the psychological methods employed are justifiable, that some of them are mathematically feasible and that both the method and the statistics are of an ancillary nature.

Dr W. G. Hiscock. *Psychological tests in industrial selection*

The complicated nature of dyestuffs manufacture, due to the very wide range of colours demanded and the very rapidly changing fashions, necessitates a high standard of process worker. Moreover, at the Grangemouth Works of Imperial Chemical Industries, Ltd., the very expensive dyestuffs, based on the anthraquinone molecule, are made.

Formerly the method employed was to select the workers by interview by the Labour Manager and subsequent probation at the more simple operations. Although this method did yield the desired result ultimately, it was only through the unsatisfactory procedure of discharging a number of workers on whom a certain financial outlay had been made in training them. The problem was handed over to the National Institute of Industrial Psychology and a study made early in 1934. At this time the process workers were satisfactory as a result of careful selection on probation.

The investigation was carried out in consultation with the manufacturing staff, foremen and process workers, and the following list of required qualities was compiled: energy, dexterity, accuracy, time sense, disparate attention, judgement, intelligence, alertness, memory for instructions, understanding of figures, mechanical ability, cleanliness and tidiness, thoroughness and application, reliability or dependability, self confidence, equanimity, co-operativeness, interest in the work and, later, experience.

On the basis of this job-analysis a test battery was drawn up, the core of which consists in an analogous test in which the testee has to attend to five different tests distributed in different parts of the test room, a situation in many ways analogous to the manufacturing shed, where the process worker has to attend to anything up to a dozen different processes, maintaining each in the right state of temperature, pressure and so on, and adding the right quantities of the right materials at the right times.

The test battery has been described recently (Hearnshaw, 1935).

Since the summer of 1934 all applicants for work, except tradesmen, have been submitted to these tests, in addition to a medical examination. The successful applicants have been carefully nursed through a training period, during which their suitability has been followed up at regular intervals by a definite method and the results recorded against the test results.

There has not so far been enough experience to permit a statistical correlation of the test marks against subsequent performance, as it takes several years before a recruit becomes a permanent process worker. Some very definite conclusions have, however, been arrived at:

(1) The proportion accepted on test varies between 55 % in boys to 69 % in adults.

(2) No recruit who has passed the test has been discharged for unsuitability, although two have been discharged for pilfering.

(3) Those obtaining the highest marks so far appear to be the most suitable.

(4) A few accepted on appearance but not having passed the test have all been found unsuitable to proceed to process work.

The whole management of the works is at present satisfied that recruiting by vocational testing has provided the necessary type of worker.

Mrs W. Raphael. *Discussion on "psychological tests in industrial selection"*

As Sir Frederick Marquis referred in his paper to the lack of correlation between intelligence (as measured by intelligence tests) and of sales ability, the experience of the National Institute of Industrial Psychology on this subject might be of interest. Some eight years ago we introduced selection tests, including an intelligence test, into a large store. Last year we attempted to assess the prognostic power of these tests by finding the subsequent success of the assistants selected by them. We found that on the whole neither those who got very high scores nor those who got very low scores on the intelligence test made the best assistants. The satisfactory assistants had "good average" scores. Although the sample was too small for statistically reliable results, these findings were what the experienced managers expected—they are probably due to the fact that there is a minimum intelligence level below which it is difficult to be a successful assistant, and that if an individual has a very high level of intelligence she tends to become bored, and therefore inefficient, by her comparatively restricted job.

In considering the use of psychological tests in industrial selection we have, in this discussion, perhaps minimized the importance of the proper choice of tests to use. Whether psychological tests are useful in industrial selection is chiefly dependent on whether the right tests have been applied. Obviously it is unsatisfactory merely to take a random series of standardized tests—whether intelligence tests, spot-dotting tests, reaction-time tests or dynamometer readings—and apply them to people in varying occupations hoping that they will have diagnostic value. The most satisfactory method of preparing selection tests for an occupation—be they analytic, analogous or even sample tests—is to consider the aptitudes needed for success in that operation and then devise or construct tests that measure the same aptitudes, but which can be performed without previous experience.

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The electrical response of the auditory mechanism in cold-blooded vertebrates

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[Plates 24–27]

In mammals the changes of electrical potential in the ear and 8th nerve are mainly due to the sound-receiving mechanism. The nerve must also register the action of the gravity receptors and semicircular canals, but the potentials due to these are swamped by the much larger cochlear effect. In cold-blooded vertebrates, on the other hand, impulses from the vestibular organs are often more prominent than those due to sound and have attracted more attention. Thus Ross (1936) has analysed the receptors in the frog's labyrinth by recording from single fibres of the 8th nerve, and Löwenstein and Sand have studied the discharge from the semicircular canals in fish, but there has been little recent work on the electrical response to sound either in fish, amphibia or reptiles, though an early paper of Wever and Bray (1931) deals with the response in the terrapin.

The present observations have been made on the alligator, the tortoise, the frog and the eel. These were chosen to offer a wide range of structure both in the middle and inner ear. A limited survey of this kind is not of much value from the point of view of comparative physiology, for there are many other species which must be investigated if we are to follow the development of the sound-receiving apparatus in the vertebrate. None the less, the results seem to be worth putting on record since they have some bearing on the general problem of the cochlear potentials and of the mechanism of the sensory endings.

METHOD

In all the animals the exposure of the nerve is a simple matter. The head is cut off, the skull opened and the brain destroyed to the level of the cerebellum. The brain stem is then dissected away carefully, leaving the 8th passes through a tunnel in the quadrate bone (which forms the inner wall of the tympanic cavity) and then through the sinus pericapsularis to the inner ear. Here it expands into the stapes which plugs the opening of the cisterna perilymphatica. The sensory endings of the papilla basalis in the saccule are immediately opposite the stapes. About 30% of the tortoises examined have had a solid otolith in the saccule: in the rest there was merely a collection of chalky particles as in other reptiles.

The general performance of the tortoise's ear at a fairly low temperature can be seen very well from records of the nerve response to speech. Those in fig. 2A show the action potentials in the auditory nerve when the phrase "good morning" is spoken very softly, with a rising intonation, about 3 ft. from the ear. The response to the complex train of sound waves is extremely simple. It consists of a regular series of large potential waves rising in frequency from 85 to 120/sec. Fig. 2B is the response to the phrase "how do you do?" spoken softly with a falling intonation: the frequency of the response varies over much the same range and there is little to distinguish the two records except the timing of the groups of waves.

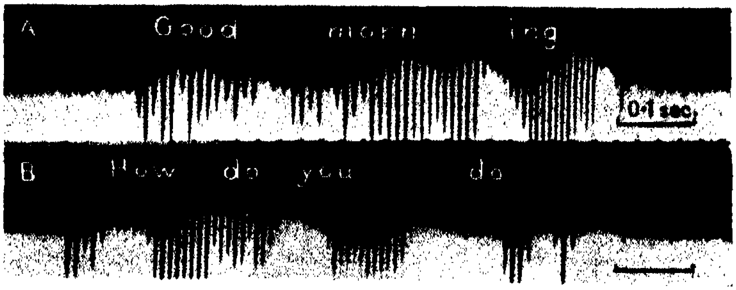


Fig. 2. Action potentials in 8th nerve of tortoise in response to speech (A, "Good morning"; B, "How do you do?") showing limited frequency range of potential waves. Records made with Matthews oscillograph, potential waves about $50\mu V$. Time given in these and following records by line marking 0·1 sec.

When the ear is tested with pure tones of variable frequency and intensity the simple character of these records is easily explained. The limited frequency range of the potential waves is merely the frequency range of the sounds which are most effective in stimulating the nerve. The nerve will respond rhythmically to a 100-cycle note which is not far above the threshold for the human ear, but a note of 200 cycles must be much louder to be effective. Thus the response to any sound with a component in the 100-cycle range will be mainly a train of potential waves at that frequency, although a pure tone at 200 cycles, if loud enough, would give a 200-cycle response.

Fig. 3 gives a series of curves illustrating the point. They show the threshold-frequency relation in four preparations at room temperature (about 13° C.). The stimuli were pure tones produced by a loud speaker and the intensity measurements were checked by a microphone placed in the same position as the preparation. The threshold is taken as the least intensity needed to produce a rhythmic series of potential waves in the nerve at the same frequency as that of the stimulus. Responses at half or double the stimulus frequency have not in fact occurred: sounds of high frequency may produce an irregular discharge in the nerve, but the present curves apply only to the rhythmic effect. The intensities are given in decibels above the intensity of a 100-cycle note just audible to the human ear (E.D.A.). Thus at 100 cycles the threshold for the tortoise's ears is 20–25 decibels above that of the human ear. At 200 cycles the difference would be greater than appears from the slope of the curve, for the human threshold is lower at 200 than at 100 cycles (cf. fig. 18). The tortoise's ears become distinctly more sensitive if the hard external skin over the ear drum is removed (cf. fig. 4A) or if the temperature is raised to 20–25° C.

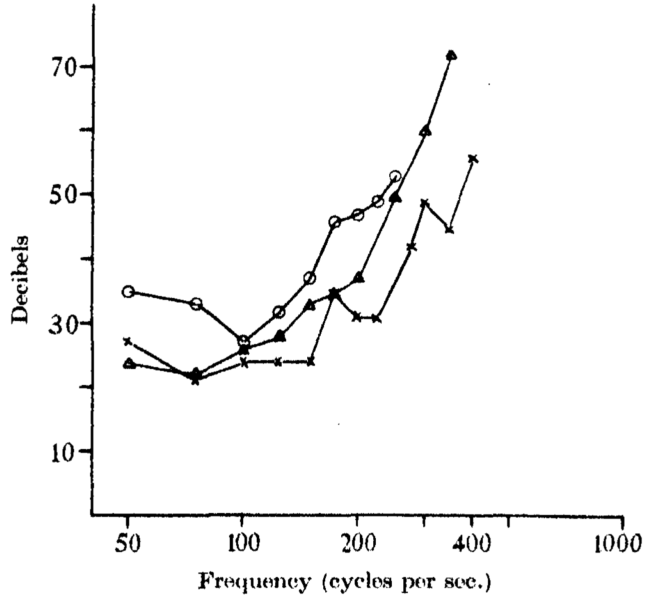


Fig. 3. Threshold-frequency curves showing the intensity of sound needed to give a nerve response in three tortoises at (low) room temperature. Intensities are given in decibels above the audibility threshold for E.D.A. at 100 cycles.

The limited frequency range for high sensitivity was thought at first to be due to middle ear resonance, i.e. to some part of the receiving mechanism having a natural period in the region of 100 cycles and so tending to vibrate more readily in response to sounds of this frequency. To test this suggestion attempts were made to alter the form of the threshold curve by removing part of the ear drum, attaching weights to the drum or columella, or restricting the movement of different parts. It was found possible in this way to modify the general sensitivity of the ear and to introduce various peaks and troughs into the threshold-frequency curve: for instance, fig. 4B shows the effect of fastening a heavy clip to the oral margin of the drum. But although this treatment has evidently produced various resonances in the middle ear mechanism, it has not changed the general form of the curve and the sensitivity is still greatest in the region of 100 cycles.

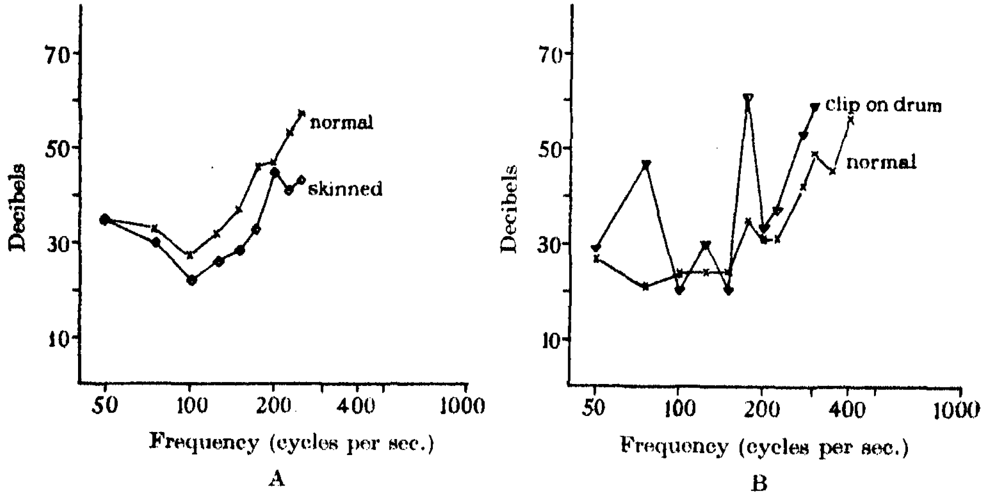


FIG. 4. Threshold-frequency curves for nerve response showing: A, Increase of sensitivity produced by removing the skin covering the ear drum. B, Effect of loading the head of the columella with a metal clip. The general form of the curve is unaltered in spite of the troughs and crests which are introduced.

Since interference with the middle ear did not produce more obvious changes in the curve, it seemed likely that its form was determined not by the mechanical properties of the middle ear system but by the capacity of the nerve endings and nerve fibres to react to stimuli of different frequency. In a frog's motor nerve fibre an effective stimulus is followed by an absolute and then a relative refractory state, and the excitability does not regain its normal level until about 0·01 sec. has elapsed. Thus a series of stimuli of the threshold strength for resting nerve might be effective if repeated at 100/sec., but at any higher frequency the nerve fibre would not have time for complete recovery between successive impulses and stronger stimuli would be needed to excite. It follows that the curve relating frequency of stimulation and threshold strength for a nerve fibre excited electrically would have the general form shown in fig. 5, remaining level for frequencies up to about 100/sec. and then rising more and more steeply. Clearly the rise in the threshold for sound in the tortoise's ear might be due in the same way to the need for stronger stimuli when the frequency becomes too high to allow complete recovery between the impulses.

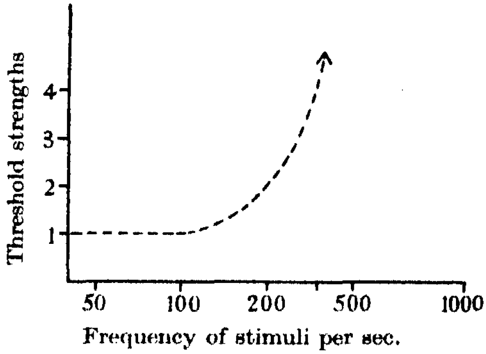


FIG. 5. Hypothetical curve showing threshold-frequency relation for frog's nerve stimulated rhythmically. It is assumed that the recovery of excitability is complete 1/100 sec.

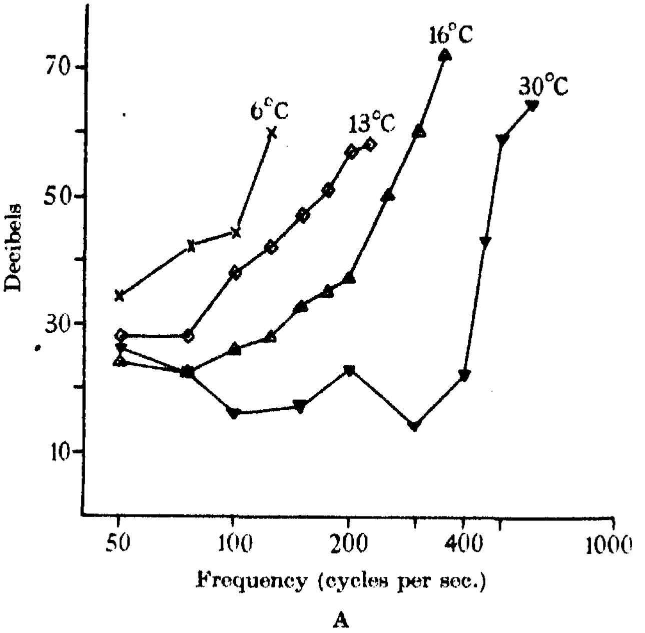
The simplest method of testing the point is to alter the temperature. A fall of 10° C. will cause a threefold increase in the recovery time of a nerve but would be most unlikely to have such a large effect on the mechanical properties of the middle ear. Thus the threshold-frequency curve should be greatly changed by temperature if it is dependent on the properties of the nerve endings, but not if it is dependent on the middle ear. Figs. 6A, B give a series of curves made from two preparations at various temperatures. It will be seen that the rise of threshold occurs over quite different frequency ranges at low and at high temperatures. At 6° C. the maximum frequency of sound to which the nerve will give a rhythmic response is only 125/sec.; at 16° C. it is about 350 and at 30° C. 500/sec. Since this implies a temperature coefficient of the same order as that of the recovery time of a nerve fibre, it seems highly probable that the rise of threshold with sounds above 100 cycles is due to the incomplete recovery of the nerve endings and not to a fall in the efficiency of the middle ear.

There is, however, a more direct line of evidence. It consists in determining the efficiency of the middle ear at different frequencies by recording the inner ear potentials, the equivalent of the cochlear potentials in the mammal. This cannot always be done: in most of the land tortoises the potential changes which could be detected by a moist thread electrode on the wall of the inner ear were too small to be distinguished as genuine biological effects, but in the two box tortoises which gave the curves in fig. 6 the wall was thinner and there were definite oscillations of 10–20μV amplitude, resembling the sound waves to which the ear was exposed. The oscillations were not present when the electrode was moved to some other part of the cranial wall. Records of these inner ear potentials and of the action potentials in the nerve are given in fig. 7, Plate 24, to show the different form of the waves.

Whatever their origin may be, the inner ear potentials are clearly an index of the performance of the sound-receiving mechanism, and they show that this mechanism can vibrate at rates as high as 700–800/sec. The curve (fig. 8A, B) relating the frequency of the sound and the intensity needed to produce a definite potential oscillation in the inner ear is quite unlike the curve for the nerve response. It falls gradually towards a plateau at 200–400 cycles and rises to a limiting value at about 800 cycles. A change of temperature of 20° C. has very little effect on the general form of the curve, though the sensitivity is increased slightly by warming the ear.

It follows that at temperatures as low as 7° C. where the nerve response goes no higher than 125/sec. the middle ear can still transmit vibrations of 500–600 cycles to the inner ear, and that its efficiency is just as great at 500 cycles as at 100. It is the nerve endings and nerve fibres which cannot transmit such high frequencies, since they must have a finite time for recovery after each impulse. Presumably the threshold curve for the nerve response begins to rise as soon as the interval between successive stimuli is too short to allow complete recovery. At a low temperature, therefore, the nerve endings take 0·01 sec. or more for complete recovery; at high temperatures (25–30° C.) recovery must be complete in 0·003 sec. or less, for then the rise in threshold does not begin until the frequency reaches 300–400 cycles. The much wider frequency range of the nerve response at high temperatures can be seen in fig. 9, Plate 24, which gives the response to speech ("Good morning") with the preparation at 16° and 27° C. The latter is a much more faithful rendering of the complex sound waves, though it is not faithful enough to be converted back again into intelligible speech.

It must be remembered that the curves in figs. 3, 4 and 6 show the intensity of sound needed to produce a rhythmic nerve response at the same frequency as the sound. When the sound is of too high a frequency to give such a response it may none the less excite the nerve endings intermittently,



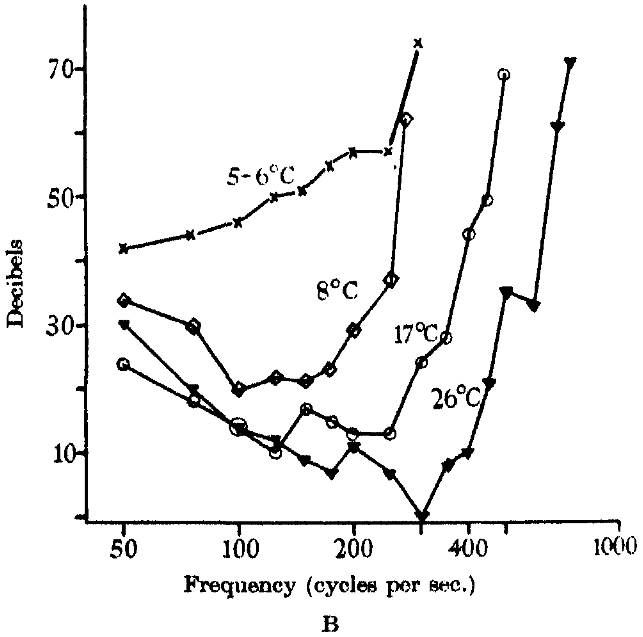


FIG. 6. A, Threshold-frequency curves for the rhythmic nerve response in a box tortoise at different temperatures. The range of response is greatly increased by a rise of temperature. B, Another preparation.

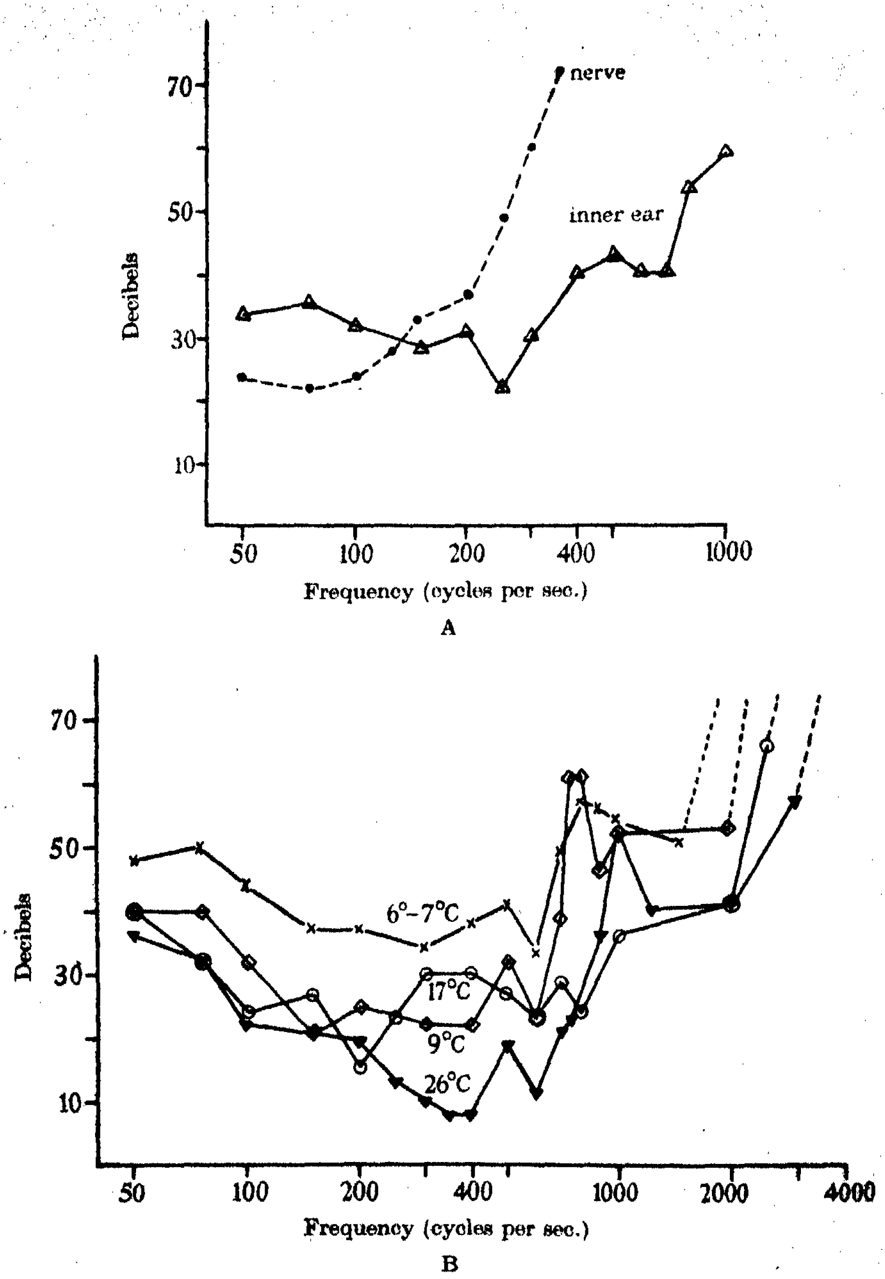


FIG. 8. A, Threshold-frequency curve for inner ear potentials compared with curve for nerve response. Box tortoise at 16° C. B, Threshold-frequency curves for inner ear potentials at different temperatures. Box tortoise. Compare with fig. 6A, B.

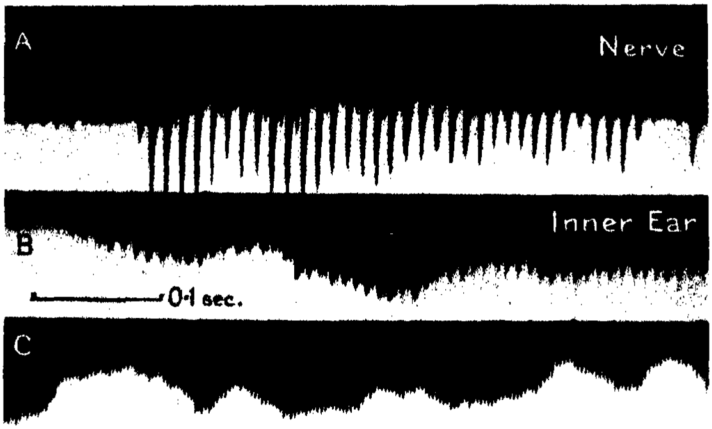


FIG. 7. Potential changes, A, from the 8th nerve, and B and C, from the inner ear of a box tortoise in response to voice sounds. In A and B, the stimulus is a note of low pitch ('ah'). The nerve response shows only the fundamental; the inner ear response gives higher frequencies as well. In C, the inner ear potentials have a frequency of 340 a sec.

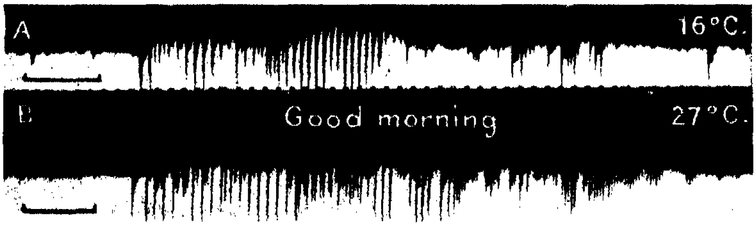


FIG. 9. Nerve response at different temperatures to a complex sound. Speech—'Good morning'—with preparation at 16° and 27° C. At 16° C. the higher frequencies are absent.

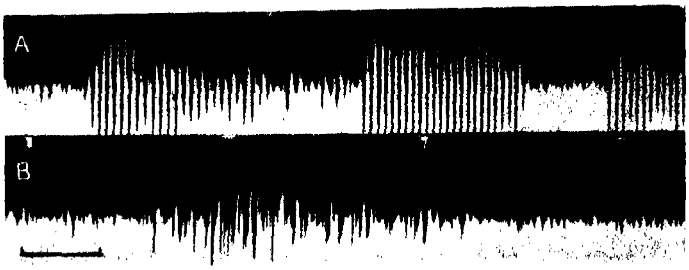


FIG. 10. Action potentials in 8th nerve of tortoise, A, in response to sound; B, in response to rotation in a horizontal plane.

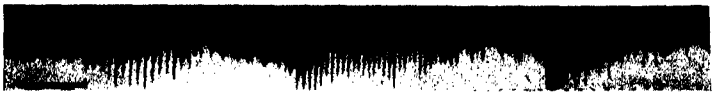


FIG. 11. Action potentials in 8th nerve of frog (*Rana esculenta*) in response to loud sound—'Good morning' spoken loudly at a distance of one foot from the ear.

giving an irregular succession of potential changes which are of small amplitude because they do not occur synchronously in all the fibres. Again at moderately high frequencies a loud sound may be needed to give the rhythmic response and the threshold for the irregular response may be lower. Accurate threshold measurements for the irregular response have not been made, since it develops gradually as the sound is increased. It should be emphasized, however, that the nerve response which we are recording is the product of a large number of nerve fibres and cannot always be taken to show how the stimulus affects the individual nerve endings. To make sure of this it will be necessary to work with preparations in which only one nerve fibre is in action. There should be no great difficulty in doing so, but meanwhile there is little to be gained by further discussion of the composite effect.

Response to vibration, etc. When the middle ear is intact, a slight movement of the stand will often set up a train of potential waves in the nerve with a frequency round about 100/sec. When the drum is removed the response to sound is lost, but vibrations of larger amplitude will give a response at the frequency of the vibration, and the ear can be made much more sensitive by fixing a small lump of plasticine on the projecting stalk of the columella. With the columella loaded in this way the ear may be so sensitive to mechanical disturbance that it gives a constant series of potential waves at a low frequency (10–40/sec.).

A continued pressure on the columella or on the ear drum gives only an initial discharge. This is made up of large potential waves (0·1 mV or more) and so presumably is due to a series of ripples in the perilymph, each stimulating a large number of nerve endings simultaneously. In the discharges from the gravity receptors and semicircular canals the potentials are smaller and more irregular, for in these there will be nothing to make the nerve endings act in unison (fig. 10, Plate 25).

(*b*) *The frog*

Judged by the response in the 8th nerve the ear of the frog is highly sensitive to mechanical vibration of the supporting surface but insensitive to all but loud sounds. The sensitivity is greater in *R. esculenta* than in *R. temporaria*. In both species a slight puff of air falling on the ear drum will give a well-marked irregular discharge and speech near the ear will have the same effect if the drum is not shielded from air currents, but to obtain a genuine nerve response to sound it may be necessary to shout loudly down a speaking tube with its end a few millimetres from the ear drum, and in *R. temporaria* it is often impossible to produce any rhythmic response at all. In *R. esculenta* speech a foot or more from the ear, without a speaking tube, will sometimes give a rhythmic discharge: a continuous note from a loud speaker seems to be less effective. Since the sounds necessary to stimulate are often loud enough to cause widespread vibration of the supporting surface and of the electrodes, it is impossible to construct a curve relating the threshold intensity with the frequency of the sound. All that can be said is that there is no evidence of greatly increased sensitivity over a particular range. Indeed the response to sound, when it can be produced, seems a more faithful copy of the stimulus than the response in the tortoise, since there is less reduction on the size of the waves as the frequency rises (fig. 11, Plate 25).

As with the tortoise, the range of frequency of the sounds giving a rhythmic nerve response varies with the temperature. Fig. 12 shows the maximum response frequencies obtainable at different temperatures in one preparation (*R. esculenta*). The values agree well enough with those for the tortoise nerve at the same temperatures.

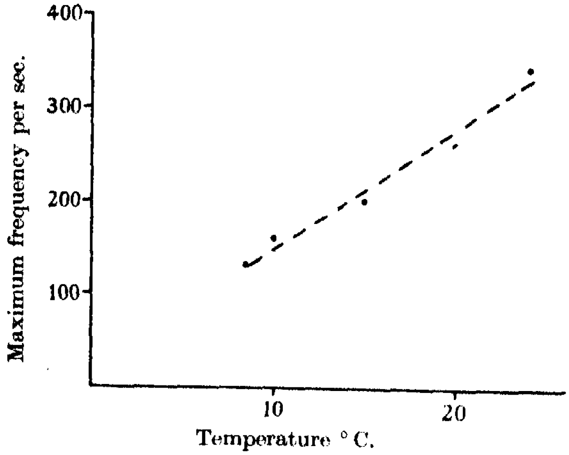


FIG. 12. Maximum frequency of action potentials produced by voice sounds in 8th nerve of *R. esculenta*. Temperature varied from 8° to 24° C.

Although the sensitivity to sound is low, that to vibration of the supporting surface is high enough to give a nervous discharge in response to very light touch with the finger tips on any part of the base board or the table on which the preparation is placed (cf. Ross 1936). Ashcroft and Hallpike (1934) have shown that the nerve endings which signal vibration are in the saccule and state that the nervous response will follow the frequency of a vibrating tuning-fork up to 512/sec. The sensitivity to vibration is not diminished by removing the ear drum, but the sensitivity to sound and to air currents is considerably reduced.

A few tests were carried out with the head arranged so that the ear drum was below the surface of a shallow pool of water. Disturbances in the water near the drum produced irregular discharges like those due to a puff of air. It was noticed that a steady current directed towards the drum gave a discharge which continued for many seconds; this, however, was probably due to eddies near the drum, for a steady mechanical pressure gave only an initial discharge. Ripples on the water give a rhythmic discharge if the ear is close enough to the surface, but loud sounds made near the surface are no more effective than they are when the drum is in the air. We must conclude, therefore, that in the water the ear mechanism may be of some value in signalling movements, but that there is no reason to suppose that it can act as a pressure or depth gauge. In the air the reaction to sound is negligible in *R. temporaria*; in *R. esculenta* loud sounds give a corresponding nerve discharge, and the sensitivity may well be greater in species with larger ear drums.

In at least one preparation (*R. esculenta*), an electrode on the wall of the inner ear has shown potential changes in response to sound at frequencies considerably higher than the limiting value for the nerve. Oscillating potentials have also been produced by vibration, but the effects have never been quite large enough to make it certain that they are not due to movement of the electrodes.

(*c*) *The alligator*

In the remaining animals, the alligator and the eel, the potentials developed in the inner ear are so large that the nerve response is often swamped by them, as it is in the mammal. The alligator was chosen because the inner ear has a definite cochlea, the Crocodiliae being the only cold-blooded animals with a structure of this kind. The middle ear is also a more elaborate structure than that of the frog or tortoise. It has a large membranous ear drum protected from injury by a fold of hard skin from which it is separated by an air space. In the specimens used in these experiments the ear drum varied from 6 to 8 mm. in diameter and the columella was about 9 mm. long. Fig. 13 is a sketch of the middle ear, opened from above, to show the attachment of the columella to the drum. Six alligators were used, five of them about 18 in. long and one 12 in. long.

Only two preparations gave records in which the nerve response could be distinguished with certainty from the inner ear potentials and with these, unfortunately, the threshold-frequency curve for the nerve response was not made. The records in fig. 14A, Plate 26, show that the nerve can give rhythmic action potentials at 250/sec. or more: at the lower frequencies there is little risk of confusing the nerve and the inner ear response, but at higher rates they are more alike and the smaller size of the potentials in the nerve makes it more difficult to decide whether they originate there or in the inner ear.

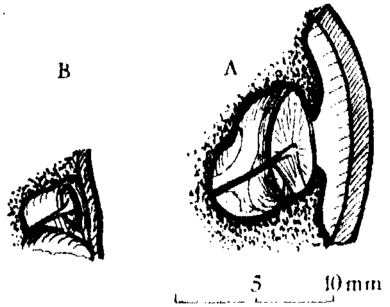


Fig. 13. A, Sketch of middle ear of alligator, opened from above to show membranous ear drum, protective skin flap and long columella. B, Middle ear of tortoise drawn to same scale.

With a direct lead from the wall of the inner ear all six alligators gave potential waves larger than those in fig. 14A, Plate 26, and a much closer copy of the sound waves. The amplitude has reached 0·5 mV; the fidelity with which the sound waves are reproduced can be seen from fig. 14B, Plate 26, and from fig. 15, Plate 26, which gives two cathode ray oscillograph records made at high speed, the upper from the ear and the lower from a microphone. Both show corresponding portions of the response to the words "Good morning", the particular sound being the final "ing". The complex wave forms are evidently reproduced almost as well by the alligator's ear as by the microphone.

As might be expected, the potential changes, like those in the mammalian ear, are a close enough copy of the sound waves to be reconverted into intelligible speech. When threshold-frequency curves are made, however, it becomes clear that the range of the inner ear response is not in fact as great as in the mammal. The threshold begins to rise at about 1500 cycles and frequencies above 4000/sec. are not reproduced (fig. 16). The sensitivity measured in this way has varied in different specimens, but has never been as great as the sensitivity of the human ear.

Conditions affecting the inner ear potentials. Although inner ear potentials (of characteristic wave form) can be recorded by an electrode on the 8th nerve or on any of its branches, their intensity is greater when the electrode leads directly from the mesial wall of the inner ear. In the young alligator this is a thin layer of cartilage: its electrical resistance would not be very high and the potentials are not greatly increased when some of the wall is removed to expose the membranous labyrinth. Extensive damage to the labyrinth abolishes the effect and minor damage weakens it. As so few animals have been examined it is difficult to say what kind of injury has the greatest effect: it can be said, however, that the opening or removal of a semicircular canal has very little effect and that a large opening into the saccule has caused an almost complete disappearance of the waves, in spite of the fact that the cochlea was left intact. It has been impossible, so far, to injure the cochlea alone, as it is awkwardly placed.

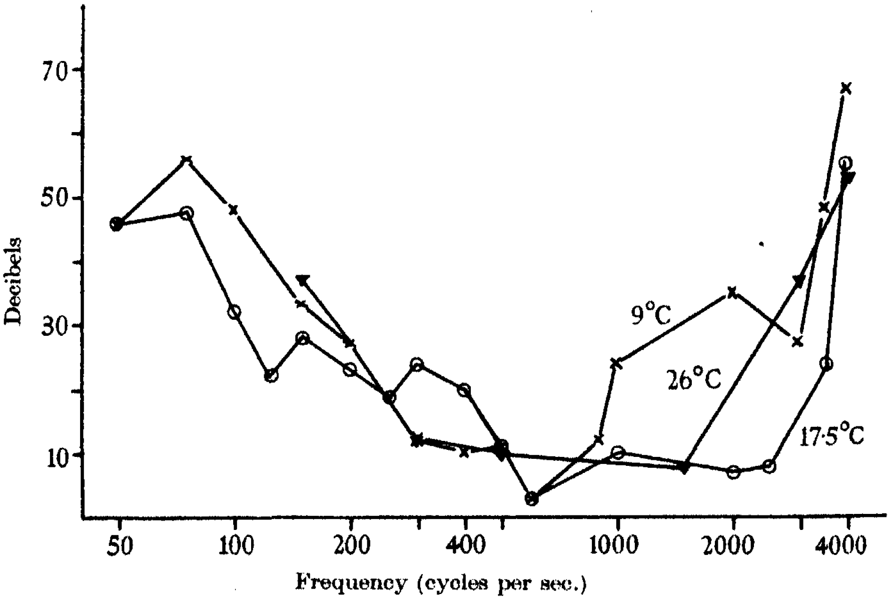


FIG. 16. Threshold-frequency curves at various temperatures for the inner ear potentials in an alligator. Same scales as fig. 3, etc.

With the inner ear intact the potential waves can be elicited at full strength for many hours after the preparation has been set up. In one preparation it was found that they had fallen to about half their initial value in 24 hours and to about a tenth in 48 hours. The fibres of the 8th nerve become inactive long before this, for a discharge in response to tilting or rotation can only be obtained for a few hours after the isolation of the head.

The effects of a change of temperature are shown in fig. 16, which gives three threshold frequency curves made from one preparation. As in the tortoise there is a slight extension of the range of response at a high temperature but no clear change in the position of maximum sensitivity. There is also no obvious change in the magnitude of the effect, although the curve does not show this.

(*d*) *The eel*

In the eel, as in many fish, there is no bony or cartilaginous wall separating the inner ear from the cranial cavity. A thin membrane encloses the perilymph and through this can be seen the sacculus with its large otolith as well as the terminal branches of the auditory nerve. There is no middle ear and no response to sounds of average intensity, but large potential changes can be set up by mechanical vibration of the head.

Potential changes in the inner ear of a fish (the pike) were recorded by Piper (1911) and it was this work which led us to investigate the eel. Piper used the string galvanometer and arranged the electrodes so that one led from the neighbourhood of the saccule and the other from an indifferent region. He found that there was usually a small difference of potential between them, the saccule being negative to its surroundings. When sound waves at 200/sec. were set up in the water this potential difference was increased and remained at the new level as long as the sound persisted. Piper considered that the increased negativity of the saccule implied an excitation by the sound, and he regarded the excitation as a continuous state since he found no fluctuations in potential corresponding to the sound waves. In this respect our results differ from his. Piper was on the look out for an oscillating potential effect; on the other hand his string galvanometer was working at high sensitivity (5 cm./mV) and the string movement would not have been rapid enough to avoid some persistent deflection in response to a series of brief currents repeated 200 times a sec. At all events the potential changes which we have found in the eel do fluctuate with the vibrations which produce them, although there is often a persistent shift in the base line as well.

A record of the response to tapping the base board is given in fig. 17A, Plate 27, and fig. 17B, Plate 27, shows a rhythmic oscillation due to a loud sound coming from a speaking tube close to the preparation. As in Piper's experiments the saccule, or rather the electrode which leads from the inner ear in the region of the saccule, develops an increased negativity with respect to more distant points. The potential changes, which are of the order of $\frac{1}{10}$ mV, appear to follow the contour of the vibration whatever its form, e.g. whether it is produced by touching the base board, scratching it, blowing on the side of the head, etc. Similar changes, of equal intensity,

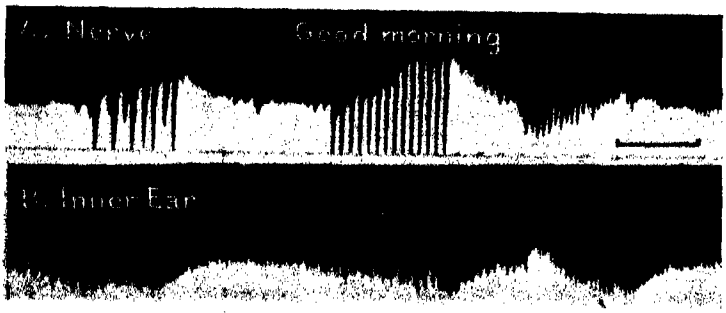


FIG. 14. A. Action potentials in 8th nerve of alligator in response to speech sounds, 15° C. B. Inner ear potentials in response to the same words. Much higher frequencies are reproduced. Sensitivity of recording system 1/5th of that in A.

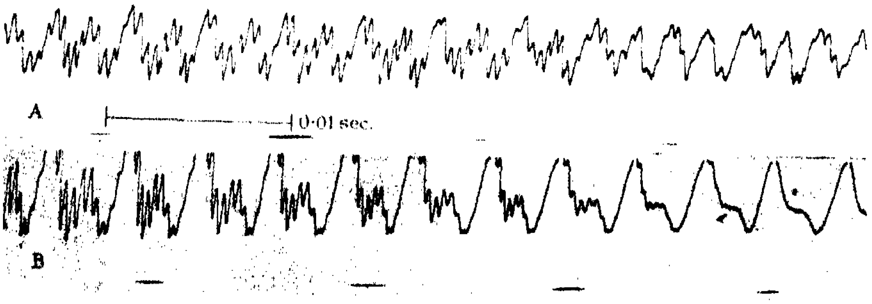


FIG. 15. A. Cathode ray oscillograph records of inner ear potentials (alligator) in response to speech 'Good morning'. The potential waves are those due to the final 'ing'. B. Records of the same sounds made with a crystal microphone having a level response (to 1½ db.) from 50–10000 cycles. Time marker gives 1/100 sec. intervals.

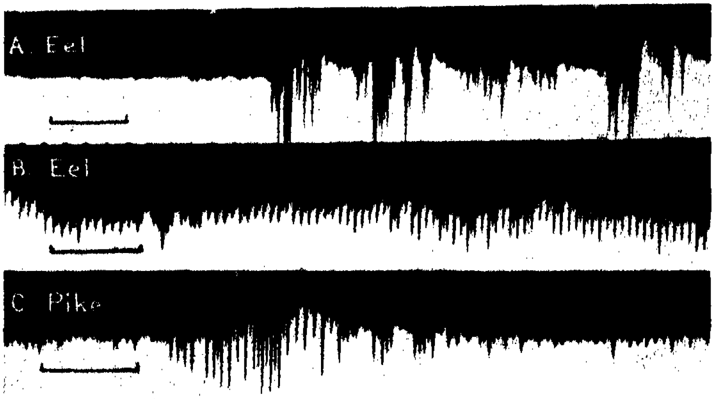


FIG. 17. A. Inner ear potentials in the eel in response to vibration (tapping base board). Owing to condenser coupling of amplifier, slow potential changes are not shown. B. Potentials in response to loud voice sounds from a speaking tube close to the head (in the air). C. Inner ear potentials in the pike in response to voice sounds (fairly loud).

can be recorded when the electrode leads from the 8th nerve instead of the saccule, though with this arrangement the sign of the deflections has sometimes been reversed, the nerve electrode becoming positive instead of negative to the indifferent one. As with the alligator the spread of the inner ear potentials into the nerve makes it difficult to obtain a true record of the nerve response.

Inner ear potentials like those in the eel have been found in the pike, goldfish and catfish. Fig. 17C, Plate 27, shows the response in the pike to voice sounds.

With mechanical vibration as a stimulus there must always be some vibration of the electrodes, and this raises the possibility that the potential changes are due to electrode movement and are not generated in the ear at all. Vibration can certainly produce small potential oscillations in a system comprising merely the electrodes and a bridge of cartilage or moist filter paper: yet the potentials from the saccule or nerve are much larger, they have a definite distribution in the region of the ear and they are greatly diminished by opening the saccule. There is little doubt, therefore, that they are due to vibration of the inner ear structures and are to that extent genuine. They do not show what information the ear can send to the brain, for without records of the impulse discharges it is impossible to say this. They do show, however, that the cochlea is not the only one of the inner ear structures which can give rise to large potential changes and presumably they have as much, or as little, biological significance as the cochlear effect.

DISCUSSION

The present results have dealt with two distinct phenomena, the potential changes developed in the inner ear and those developed in the fibres of the 8th nerve by the passage of impulses along them. The former seem to be related in a very simple way to the vibrations which produce them. They follow the contour of the mechanical changes with great fidelity, they persist for many hours after the ear has been isolated and the circulation cut off and they are very slightly affected by changes of temperature. To produce them the cochlear structure is not essential, but they are abolished by a widespread destruction of the membranes separating endolymph and perilymph. These findings agree very well with the hypothesis put forward by Hallpike and Rawdon-Smith (1934) to explain the mammalian cochlear potentials (the Wever and Bray effect). They suppose that it is produced by the movements of a polarized membrane which would be caused to vibrate by the sound waves transmitted to the inner ear, and would alter the distribution of electric forces in its neighbourhood. In the mammalian ear the membrane of Reissner might well act in this way. In the animals without a cochlea which we have examined the corresponding membrane would be that part of the wall of the sacculus or lagena which can move to and fro when a vibration occurs. The magnitude of the potentials which could be recorded as a result of the movement would depend on the area of the membrane, the distribution of fluids round it, the position of the electrodes, etc. Thus the smallness of the effect in the frog and tortoise might be due either to a smaller polarization of the membrane or to some structural detail causing, e.g., a greater degree of short circuiting.

We have found no clear evidence as to the importance of the saccular otolith. The presence of a large otolith in fish where the middle ear is absent suggests that it may be a more primitive mechanism for enabling vibrations to stimulate the nerve endings. But the actual position of the otolith in the saccule does not seem to affect the potential oscillations developed there, for these do not change appreciably when the head is placed with the lateral surface uppermost instead of the dorsal or mesial surface. Further work on the saccular potentials will be needed to show whether they are due to movements of a polarized membrane or to some part of the nerve ending structures; and whether the nerve endings are stimulated directly by movement, or by the electric currents generated by the movement or in some other way. We have not collected evidence on these points, though some is already available from the mammalian cochlea (Derbyshire and Davis, 1935*a*; Zotterman, Y., 1935; Gersuni and Volokov, 1937).

Since the potential waves in the inner ear are an index of the mechanical vibrations taking place there, they can be used, like the cochlear potentials in the mammal, to measure the efficiency of the middle ear in transmitting sound vibrations. A comparison of different ears is shown in fig. 18, which gives the threshold-frequency curves for the inner ear potentials in the tortoise and alligator, together with a curve for the cat's ear, taken from the data given by Derbyshire and Davies (1935*b*) and an audibility curve for man. As might be expected, the alligator's ear with its membranous ear drum sunk below the surface is a better receiving mechanism than the tortoise's, at least for high frequencies; but the long and rather flexible columella probably accounts for the fact that its range is considerably less than that of the mammalian ear.

A comparison of the responses in the 8th nerve emphasizes the great advantage which the mammal derives from its constant and high temperature. In hot weather the auditory nerve fibres of the tortoise and alligator should be able to transmit impulses at frequencies as high as 500/sec., but on a cold day the nerve would give much less information, for its fibres will not be able to signal frequencies higher than 100–200/sec. In the alligator it is conceivable that pitch discrimination, if it exists, might depend partly on a selective stimulation of different regions of the cochlea by notes of different frequency; but even so, the restriction of the nerve fibre response by a fall of temperature is bound to increase the difficulty of distinguishing the pitch and loudness of a sound. In the tortoise, where there is no cochlea, it is very unlikely that different parts of the nerve ending region can resonate to different notes: if the pitch is to be signalled it must be by the frequency of the nerve impulses and not by the position of the nerve fibres transmitting them.

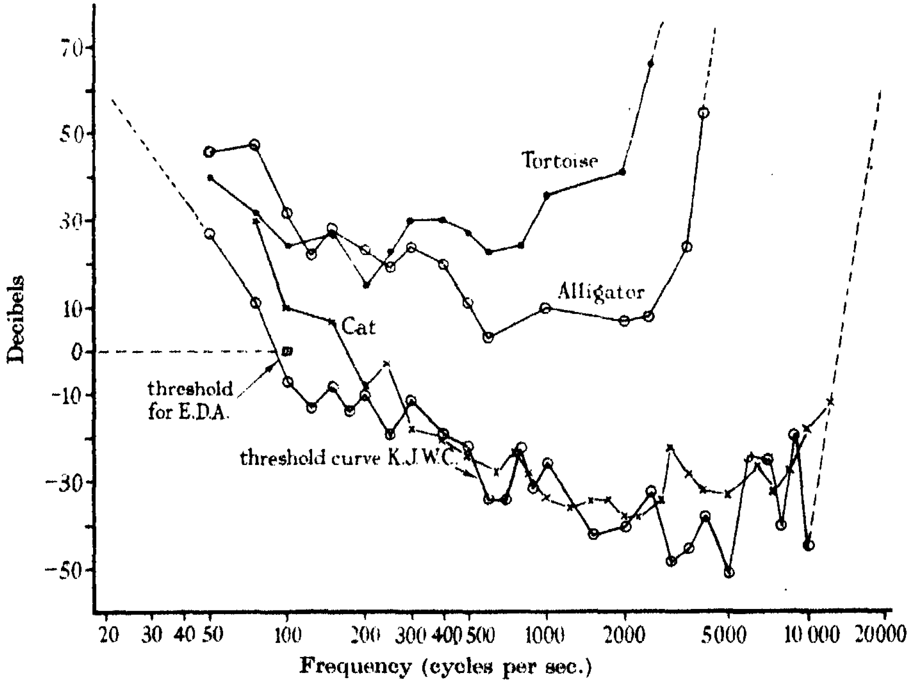


FIG. 18. Threshold-frequency curves for inner ear potentials in tortoise, alligator and cat (reconstructed from data given by Derbyshire and Davis), and audibility curve for man (K. J. W. C.). All curves to the same scale. The threshold at 100 cycles for E. D. A. is also given.

Whether these animals do, in fact, discriminate the pitch of the sounds they hear is not to be decided by records of the sensory discharge without reference to the reactions which it produces in the central nervous system. As regards the signalling of intensity, it may be said that the range of variation in size of the nerve trunk response is not very great. In the tortoise the potential waves set up by the loudest sound available are not more than five times as large as those set up by a sound of threshold intensity. But with this result, as with many others, the full analysis must be postponed until records made from the whole nerve can be supplemented by records from the individual nerve fibres.

CONCLUSIONS

1. Mechanical vibrations transmitted to the inner ear of fish and reptiles produce oscillations of electrical potential which are a close copy of the mechanical effect. They can be detected in the isolated preparation for several hours after removal from the body but are abolished by opening the saccule.

2. These inner ear potentials (the equivalent of the Wever and Bray effect in the mammal) may be used to determine the effect of the middle ear structures in transmitting sound waves.

3. Of the animals examined the tortoise and the alligator give an inner ear response to sounds which are not much above the audibility threshold for the human ear. At high frequencies, however, (above 1000 cycles for the tortoise and 3000 cycles for the alligator) the threshold rises steeply. In frogs and in fish (in the air) much louder sounds are needed to produce an effect.

4. Records from the auditory nerve of the tortoise show action potentials corresponding to the sound waves up to frequencies of 200–300/sec. The upper limit varies with the temperature. Below 15° C. the threshold for the nerve response is lowest for sounds of about 100 cycles and rises as the frequency is increased. The rise is probably due to the incomplete recovery of the nerve endings and fibres, for an increase of temperature enlarges the range of the nerve response though it has little effect on the inner ear potentials.

5. In the frog and alligator the nerve response to sound resembles that in the tortoise, though precise measurements are more difficult owing to the low sensitivity of the frog's ear and to the large inner ear potentials in the alligator.

6. These results emphasize the value of a constant high temperature for the nervous mechanism of the ear, and for any group of receptors concerned in signalling frequencies of vibration.

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Investigations of the mechanism of the transmission of plant viruses by insect vectors

II. The part played by puncture in transmission

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(*Communicated by F. T. Brooks, F.R.S.—Received* 26 *January* 1938)

[Plates 28, 29]

In an earlier paper (Storey 1933) I showed that, after *Cicadulina mbila* Naude had fed on a source of the maize streak virus, this virus appeared externally to the intestine in the insect's blood. The next stage, it may be supposed, is the collection of the virus at some centre whence it becomes available for ejection into a plant. It seems hardly possible to doubt that this centre is the salivary gland, and that the saliva is the vehicle whereby the virus is transferred into the plant. Nevertheless, my attempts to demonstrate a virus in the saliva have met with almost uniform failure.

The problem of the mechanism of inoculation is thus not simply solved. As one step towards its solution, I now present evidence bearing upon the factors that decide whether an insect succeeds in causing the infection of the plant.* In a later paper I shall consider the process of excretion of saliva and attempt to relate the facts so obtained to the conclusions of the present studies.

MATERIALS AND METHODS

The virus used in these studies was a strain of the streak virus highly virulent to maize, maintained in the greenhouse by repeated transfers over several years. During this period no marked deviation from the expected symptom manifestation was shown by any plant to which it was transferred (unless the temporary infections referred to in the footnote on p. 465 are to be so interpreted). There is no evidence to suggest that the original virus was a complex or that variant strains had arisen during the course of the work. Even if small variations had occurred, the design of my experiments would ensure that the results were not influenced thereby.

Adult insects from pure *active* races (Storey 1932) of *Cicadulina mbila* Naude were alone used. "Infective" insects were obtained, unless otherwise stated, by allowing active insects to feed on whole diseased plants for at least 4 days. The experimental plants were raised in gauze-protected houses from seed of a white dent type of maize, taken at random from a bulk sample. Usually twenty-four seeds were set in a single box of soil, and the seedlings were used as soon as the first leaf was fully developed. In all comparative experiments the methods adopted ensured random sampling of the insects and an even distribution of treatments between separate boxes; but, in order to simplify procedure, the treatments were not randomized for the position of the plants within each box. This precaution appeared unnecessary in view of the uniformity of conditions obtained by growing all the plants in a single prepared block of soil; but on the advice of Mr F. Yates this further precaution was observed in my latest experiments. In judging the significance of most of my results, however, I have disregarded this possibility of error.

Unless otherwise stated, the lower surface of the first leaf was exposed to the insect, which was confined in a small leaf cage of the type already described (Storey 1928). Short contacts* (i.e. of one or two hours) were timed from the moment when the individual insect alighted on the leaf, and the actual period during which the insect was resting on the leaf did not differ from the set period by more than one minute. At the end of the contact, the insect was removed without injuring the leaf. The contacts of all insects in comparative series were made as nearly simultaneous as possible, by starting them in as rapid succession as manipulation would allow. Since hungry insects show less tendency to wander from the leaf, the experimental insects were usually starved for several hours before an experiment. Modification of this standard technique will be described in the appropriate sections.

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* I wish to emphasize a distinction in my use of the two words "inoculation"—the introduction of the virus into the plant—and "infection"—the inception of the disease process, which ultimately results in the appearance of symptoms. Theoretically inoculation may or may not be followed by infection. On this definition it is illogical to use "infective" for describing an insect that is capable of successfully inoculating a plant; but it is the common usage and there is no preferable alternative.

EXPERIMENTAL RESULTS

How variations in the insect and the plant may influence infection

Two factors may determine whether a contact of an insect vector will result in the infection of a plant: (1) the ability of the insect to inoculate a dose of virus within the time allowed, and (2) the ability of the plant to resist infection by the dose inoculated. Although an active *Cicadulina mbila*, after it has fed on a source of the streak virus, will normally never fail to cause infection of a maize plant when the period of contact is prolonged (Storey 1932), it will often fail when the contact is short. The purpose of the work now described was to decide whether success and failure could be attributed to variations in the insects' inoculative ability or in the plants' susceptibility.

The essential feature of this problem is that each individual insect and plant is unique. Study of the insect presents no difficulty, for we may estimate its inoculative ability over a chosen period of time by allowing it to make contacts with a succession of plants. If the plants vary in susceptibility, chance alone will determine whether at each contact the insect meets a plant of high or low susceptibility. Several series of experiments have been performed on these lines. For example, random samples of ten male and ten female infective insects were tested in eleven successive 1 hr. contacts on a single day. Individual insects caused infection of from one to eleven plants. The χ^2-test applied independently to the results from each sex group demonstrated a marked deviation from the expected binomial distribution. Three other experiments on somewhat similar lines all gave values for χ^2 that would be realized by chance less than once in a hundred trials. These results thus demonstrate that infective insects do vary in their inoculative ability; and that the outcome of a contact of an insect with a plant is in part determined by an inherent quality in the insect, which I term its *potency*. Apart from an indication that females tend to be more potent as a class than males, I have been unable to discover any factors that control potency or to obtain more uniformly potent insects by any treatment.

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* The reader will note that throughout this paper I infer into the words "contact" and "puncture" a sense of duration. "Contact", as here used, means a maintained position on the leaf; "puncture" a maintained inserted position of the mouthparts at a single site.

The susceptibility of the plant cannot be studied by the procedure of replicated trials, for the individual plant can be used but once. The only approach to the problem that I have been able to devise is by a somewhat devious route. If plants be exposed to short contacts of single insects, some of the plants, as we have seen, will be infected and some will survive. Let us assume that the outcome of a contact is determined solely by the insect's potency. If now the insects be combined into groups, the effect exerted by a group will, on the simplest analysis of the problem, depend on the potencies of the individual members constituting the group. The probability of infection by a group will thus be the probability of its containing one or more members that would alone have caused infection. This probability can readily be calculated; if p and q be the probabilities of success and failure by single insects, then q^n is the probability of failure by a group of n insects, and $(1-q^n)$ the probability of success.

In the preceding argument, I assume that the effect produced by one insect is independent of the effects produced by any other insects in contact with the plant. This hypothesis of independence has recently been briefly discussed by Watson (1936) and has been accepted by her as according with the results of transmission experiments with an aphid, *Myzus persicae* Sulz. It is possible however that the effects of the members of a group might not be independent. In the first place, their effects might, as Watson notes, be cumulative. That this can occur is implied in the presentation by Carsner and Lackey (1929) of their mass-action hypothesis. The only application that such an hypothesis can have to my present problem is that groups, containing no insects that would alone have caused infection, may yet cause infection by a cumulative effect; that is, the probability of failure by a group will be *less* than q^n. The alternative possibility is that the members of a group may exert an inhibitory effect on one another, so that the probability of failure by a group will be *greater* than q^n.

The foregoing argument depends on the assumption that an insect's potency is the sole variable determining the outcome of its contact; that is, that the insect always exerts its effect on plants of uniform susceptibility. If the plants vary in susceptibility, the results of experimental trials will not accord with the frequencies calculated on any of the hypotheses considered.* The difficulty cannot be overcome by any system of randomization or replication. This is most clearly seen by considering, as does Watson, the limiting case where a proportion, Q, of the plants are entirely immune. However large a group of insects may be, it can never cause infection of the immune plants; so that, while the calculated value of q^n will tend towards zero as n is increased, the proportion of surviving plants can in fact never fall below the value Q. In general, if the plants vary in susceptibility, the number of failures realized from a group of insects will tend to exceed the calculated number, as the size of the group is increased. Experimental trials may therefore indicate whether variation in susceptibility of the plants exists.

TABLE I. INFECTIONS OBTAINED FROM 1 HR. CONTACTS OF SINGLE *CICADULINA MBILA* AND GROUPS OF TWO, THREE AND FOUR INSECTS

(1) Series no.	(2) Size of group tested	Results from single insects		Results from groups		(7) Calculated value of q	(8) Calculated value of χ^2 ($n = 1$)	(9) Significance of deviation from expectation
		(3) Positive	(4) Negative	(5) Positive	(6) Negative			
1A	2 insects	25	55	36	44	0·7215	0·739	Not significant
	Expectation	*22·28*	*57·72*	*38·36*	*41·64*			
1B	2 insects	33	47	48	32	0·6142	0·418	Not significant
	Expectation	*30·86*	*49·14*	*49·82*	*30·18*			
2	3 insects	27	50	47	29	0·7005	1·445	Not significant
	Expectation	*23·06*	*53·94*	*49·88*	*26·12*			
3	4 insects	30	50	55	25	0·7117	4·250	Significant
	Expectation	*23·06*	*56·94*	*59·48*	*20·52*			

In Table 1 appear the results of experiments comparing the infections from 1 hr. contacts of single insects with those from groups of two, three and four insects. The numbers shown are the total successes and failures in eighty tests, made up of eight replications on successive days of ten tests each of single insects and groups. The experimental technique was as follows: the insects were distributed singly into leaf cages, and the necessary numbers of cages, selected at random, were clipped on to a leaf of each plant. The contact of each insect was timed in the manner already described. For testing the four-group only, the technique was modified as follows. The tip of a leaf was introduced into a 30 mm. length of glass tubing of internal circumference slightly greater than the width of the leaf. Wool plugs closing each end pressed the leaf into contact with the inside of the tube. Insects introduced by pipette, were bound to settle at once on the leaf, since it almost completely lined the cage in which they found themselves. The same technique was here used for the control plants receiving single insects.

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* I am indebted to Mr F. Yates for directing my attention to this point and for assistance with the statistical treatment of the experimental results.

The maximum likelihood values of q for each experiment appear in column (7) of Table I, and from these the expected proportions of successes and failures on the independence hypothesis have been calculated. The values of χ^2 shown in column (8) do not individually suggest a deviation from the hypothesis greater than might be encountered by chance, except in the last experiment. Throughout, however, there is a tendency for the number of failures from groups to exceed the calculated number, and for groups of four insects this deviation is significant. The general trend therefore cannot be disregarded.

I have already considered the causes that might lead to the number of failures realized being greater than q^n: an inhibitory interaction between the insects, or a variation in the susceptibility of the plants. In choosing between these alternatives, we may note than an inhibitory effect by an insect, that would under altered circumstances itself cause infection, seems highly improbable. If it be accepted that the results are explained by plant variations, we still cannot certainly decide between the independence and the cumulative hypotheses. But the reasonably close and consistent agreement of three experiments of Table I with the expectation on the independence hypothesis at least favours this hypothesis. So also do the results of an experiment that compared the number of infections produced in plants that received 1 hr. contacts (1) of two insects simultaneously, and (2) of two single insects successively at an interval of 3 hr. The general procedure was the same as that described earlier. There were forty-eight infections in the "simultaneous" series and fifty-two in the "successive" series, each out of a total of eighty. The difference is not significant. Now the cumulative hypothesis requires that separate contacts shall be able to exert a combined effect. The foregoing experiment compared contacts separated in *space* with those separated both *in space* and *in time*. That contacts so differently separated should exert an equal combined effect is at least improbable. On the other hand the experimental results are in entire accord with the independence hypothesis.

To summarize, I interpret my results, as does Watson hers (1936), as conforming with the independence hypothesis, while recognizing that my plants vary somewhat in susceptibility.

The part played by the plant tissue receiving the inoculation

In the course of a study of the movement of the virus in the plant, I have carried out experiments in comparing the number of infections obtained from short contacts of infective insects according to whether the exposed portion of the leaf was left intact or cut off immediately after the contact. The insect could have punctured the leaf at a point between 2 and 12 mm. above the cut. In Table II appear some typical results from 1 and 2 hr. contacts expressed as the mean successes per cent from eight replications of fifteen-plant trials, carried out on the general lines of the preceding section. In these and all other experiments more infections were obtained in the plants left intact than in those cut; but the difference was small and in only one experiment was it statistically significant. Although these results suggest a real effect from cutting, the important point for the present purpose is the smallness of the effect. It is almost immaterial whether, when the insect is removed, the tissue into which it has punctured is also removed from the plant or left uninjured. In general therefore the virus had become established in the plant and had moved down at least a few millimetres during the time that the insect was in contact; or, if the virus had not become established in the plant during contact, it would not become established after contact had ceased.

TABLE II. THE EFFECT OF CUTTING OFF THE PART OF A MAIZE LEAF EXPOSED TO THE PUNCTURES OF INFECTIVE *CICADULINA MBILA*

			Mean percentage of infections		
Exp. no.	Period of contact hr.	No. of insects	Cut at end of contact	Not cut	Significance of difference
1	1	12	55·0	68·3	Not significant
2	1	1	16·8	20·8	Not significant
3	2	1	56·4	67·0	Significant

Provided the leaf is not cut, the length of leaf through which the virus must travel downwards is without influence on the probability of infection. An experiment, of eight replications of ten-plant trials, compared the infections resulting from exposing the tips and bases of similar leaves to 1 hr. contacts. In the first series, the mean distance to be travelled before the stem was reached was 28 cm.; in the second 12·5 cm. The total resulting infections were 49 and 50. Bennett (1934) obtained similar results in the infection of rapidly growing beet seedlings but not if they were growing slowly.

General observations on puncture by the insect

Observation by microscope of many *Cicadulina mbila* led me to the following general account of their behaviour. Upon alighting on any penetrable surface the insect punctures immediately with only rarely any visible selection of position. The mouthparts may be withdrawn after a few minutes or only after several hours; but provided the insect does not move off a penetrable surface they are always reinserted within a few seconds either close to the old site, or more usually at some distance from it. Thus the contact of an insect with a leaf invariably implies a continued inserted position of the mouthparts; but during a timed contact an insect may make one or several punctures. When disturbed, the insect withdraws its mouthparts instantly and without apparent effort.

If, as appears probable, the defaecation of "honey-dew" is a process for rapidly eliminating excess water taken up in the dilute food solution, the rate of defaecation provides a guide to the rate of uptake of food from the leaf. I have observed rates as high as two droplets every minute; a more usual rate is about three in an hour; but an insect will often fail to expel any for an hour or more. Thus an inserted position of the mouthparts does not necessarily imply that the insect is sucking food from the leaf.

The punctured leaf undergoes no externally visible change either during puncture or at any stage after withdrawal. Histological preparations of the leaf, however, show, as is usual with Homoptera (cf. Brandes 1923; K. M. Smith 1926), a clear trace, in the form of a tubular sheath, of the course taken by the stylets (figs. 1–12, Pls. 28 and 29). With the gentian violet-orange G combination the sheath proper is strongly basic-staining (violet), with an irregular outer layer of acid-staining (orange) material. The termination of the sheath however may sometimes show only the orange layer, e.g. fig. 6, Pl. 28.

I defer discussion of the nature and origin of this sheath to a later paper, being here concerned only with the evidence that it provides of the path taken by the stylets. Of this I am satisfied that it provides a complete record, except that the finer details of its exact termination can rarely be made out. The stylets, taking an intracellular course, may penetrate all tissues of the leaf. New branches of the trace appear to be made periodically during the maintenance of the puncture, for punctures of short duration leave a relatively simple trace. But punctures, even though long maintained, that penetrate directly to the phloem, rarely branch (figs. 6 and 7, Pls. 28 and 29), except within the phloem (fig. 4, Pl. 28). A maintained puncture into the mesophyll between the vascular bundles has usually many branches, one of which may pass into the phloem of an adjacent bundle.

The behaviour of *Cicadulina mbila* is not typical of all jassids. The histological studies of other species by K. M. Smith (1926) and F. F. Smith and Poos (1931) reveal marked differences. My own observations of a species of *Erythroneura* show that it normally rests with its stylets withdrawn; when feeding it traverses a maize leaf, feeling the surface with the tip of the rostrum and penetrating it only through stomata or rarely between epidermal cells. It remains at each site only for a few minutes and on withdrawal leaves a group of cells devoid of green colour. The stylets penetrate only the mesophyll and bundle-sheath cells, and leave behind only a thin and indefinite sheath.

Puncture in relation to the uptake of the virus

Uninfective active *Cicadulina mbila*, previously starved for several hours, were allowed contact with a diseased maize leaf while under observation by microscope. Each insect was timed from the moment that it punctured the leaf and was removed at the end of one minute. Of fifty-three insects thus allowed single 1 min. punctures, twenty-six proved in later tests to have become infective. This experiment was repeated with the duration of the puncture reduced to 15 sec. with an error of about ± 5 sec.; eleven out of twenty-five insects became infective.

Streak disease produces on a maize leaf a well-defined pattern of green and chlorotic areas. The experiment in allowing single 1 min. punctures was repeated, but note was taken of the type of leaf tissue into which each puncture was made. If the puncture was near the margin between two areas the observation was rejected. Of thirty-one insects that punctured a chlorotic area, twenty-two became infective. Of twenty-eight that punctured a green area, none became infective. In a further experiment on these lines each insect was maintained under observation until it withdrew voluntarily, whereupon it was immediately removed. All of six insects that punctured a chlorotic area became infective, while none of twenty that punctured a green area did so. The mean duration of puncture in the second series was 18·5 min., the maximum being 56 min.

I conclude that virus, available to be taken up by the insect, is confined to the chlorotic areas. The clear-cut results now obtained remove the uncertainty attached to an earlier similar conclusion (Storey 1928), due to the imperfections of the technique then employed. Furthermore, the results of histological studies of leaves exposed to timed punctures permit a more detailed interpretation. As I report on p. 470, 1 min. punctures have never been known to penetrate as far as the phloem; whereas punctures that were prolonged beyond 2·5 min. often entered the phloem. Since a large proportion of the 1 min. punctures—and even 15 sec. punctures—gave infective insects, I conclude that the virus can be taken up from mesophyll tissue within the yellow areas of the leaf. Since none of the prolonged punctures into the green areas gave infective insects, I conclude that the virus cannot be taken up in these areas either from mesophyll or phloem.

Puncture in relation to the inoculation of the virus

(1) *The necessity of puncture for infection.* The view that an insect inoculates a virus by means of its mouthparts seems to be now universally held. Alternative suggestions have been made, however, and formal proof is perhaps not redundant. Insects, known to be carrying the virus but from which the stylet-tips had been cut, failed to cause infection of any plants, although they survived in contact with the plants for more than a day. On the other hand, insects that were denied all contact with the plant except through their mouthparts, by interposing a penetrable wax or other membrane between them and the leaf, successfully inoculated plants (see Table III, p. 467).

(2) *Observations on behaviour during experimental contacts.* Records were made by low-power microscopic observation of the behaviour of twenty infective insects during 1 hr. contacts with maize seedlings. Of these, eight caused infections. There was wide variation in the insects' behaviour during both successful and unsuccessful contacts. During successful contacts the insects punctured from one to nine times, the longest maintained puncture in each contact varying from 27 to 60 min.; the similar figures for unsuccessful contacts were 2–12 times and 21–53 min. The outstanding result obtained in this series is that three contacts, during which the insect punctured once only and maintained that puncture for the full 60 min., were all successful.

In a second experiment I attempted to obtain greater uniformity in the insects by using only females, reared on diseased maize and tested on the seventh day after the last change of skin. Ten out of twenty 1 hr. contacts were successful. There was again wide variation in behaviour within each class, the mean figures being as follows:

Successful: No. of punctures, 8·2; duration of longest puncture, 31·5 min.; defaecation, 1·6 droplets.

Unsuccessful: No. of punctures, 7·0; duration of longest puncture, 25·4 min.; defaecation, 8·9 droplets.

It will be recognized that an inevitable effect of classing the insects as successful and unsuccessful is to separate into the two classes those tending to high potency and those tending to low; so that a strict comparison of behaviour by the two classes would be valid, only if potency were determined solely by behaviour. In fact, however, none of the differences between the above means is statistically significant, not even that for defaecation. The figure for defaecation by unsuccessful insects is swelled by two insects that defaecated twenty-eight and thirty-two droplets each; if these two be omitted, the mean number of droplets is 3·6, a figure that is again not significantly different from that for successful insects. Thus potency is not a simple function of behaviour; nor is there any clear indication that any feature of behaviour can be related to success and failure, although a suspicion is raised that a very high defaecation rate may indicate some feature unfavourable to infection.

Similar observations were maintained on insects that were allowed to make only single punctures into each plant exposed to them. Either they were removed when they withdrew voluntarily, or the puncture was interrupted at the end of an hour. Of ninety-six single punctures, varying between 10 and 143 min., five only were successful, their duration being 10, 59, 60, 60 and 70 min. One puncture, also of 60 min., produced a temporary infection.* The proportion of successes is evidently very low. If, however, we consider only the punctures that lasted for 50–70 min., we find that 4(+ 1) were successful out of a total of twenty-five. This is not an unreasonably low proportion (cp. Tables I, II) since the majority of the experiments were done during the cool season (laboratory temperatures, 19–23° C.). But the failure of all of six punctures of durations between 70 and 143 min. and of all of nineteen punctures between 30 and 50 min. is difficult to explain. I do not doubt that simple contacts by unselected insects, equal in duration to the several observed punctures, would have given a higher proportion of successes. It must however be emphasized that the technique followed necessarily resulted in the selection for observation of those insects that were willing, after manipulation, to settle at once at a single site; very many insects that proved restless at first had to be rejected. This selection may have tended in some way to separate the insects with a lower average level of potency. Or again, a willingness to rest for long at the initial puncture after contact may indicate some temporary physiological state of the insect that is unfavourable to inoculation of the virus. No observable feature, however, in the behaviour of the insects during puncture could be related to success, except that, whereas the highest defaecation rate during a successful puncture was two droplets in an hour, eighty-five droplets were ejected during an unsuccessful 112 min. puncture. I suspect, but have not been able to prove, that a high rate indicates sucking from the xylem, which, as we shall see, would be unfavourable for infection.

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* A single spot appeared on the third day, but otherwise the plant was still free from symptoms at the end of 5 weeks. Insects fed on the spot became infective and transmitted the virus normally. A study of this type of recovery is in progress.

Although therefore a full interpretation of these experimental results is impossible, they at least provide conclusive proof that a single puncture into a plant is capable of causing it to become infected.

The portion of the leaf punctured by each insect gave me material from which histological preparations showing the course taken by the stylets could be prepared. The trace of one successful puncture, shown in the series of the photographs (figs. 1–4, Pl. 28), branched once in the mesophyll, and then one branch passed into the phloem, where a large mass of sheath material was deposited. A second showed a trace passing through the xylem and entering an adjacent phloem cell with only slight deposition of sheath material there (fig. 5, Pl. 28). All of the four successful puncture-traces examined were followed to the phloem.

To augment this evidence, sections were cut of four leaves that had been exposed to successful 1 hr contacts. Each showed several puncture-traces, at least one of which entered the phloem, e.g. fig. 6, Pl. 28.

On the other hand, sections of twenty-one unsuccessful punctures, each of more than 45 min. duration, showed traces of which eight certainly reached the phloem, three were doubtful, and ten certainly failed to reach the phloem. I have examined many other preparations from unsuccessful punctures and confirmed fully that penetration to the phloem can occur without infection following, e.g. figs. 7 and 8, Pl. 29.

(3) *Experimental control of penetration during puncture.* If, as appears from observation, infection depends in part on penetration of the stylets into the phloem, this should be susceptible to experimental test. Although the phloem is situated nearer to the lower (abaxial) surface of the leaf, the insect can certainly reach it from the upper surface, and comparative experiments revealed no significant difference in the infection rate from

either surface. But by interposing membranes, of paraffin or the compound "Sira" wax, between insect and leaf, the range of penetration was successfully restricted. Membranes cut 50μ thick on a microtome allowed infection from either surface; but membranes 150 and 200μ thick prevented infection from either. In two experiments with 100μ membranes, infection occurred from the lower surface but not from the upper (Table III). Two similar experiments showed no differential effect.

TABLE III. THE EXPOSURE OF UPPER AND LOWER SURFACES OF MAIZE LEAVES TO PUNCTURE BY INFECTIVE *CICADULINA MBILA* THROUGH WAX MEMBRANES, 100μ THICK

Exp. no.	Composition of membrane	No. of insects per trial	Period of contact days	Experimental trials			
				Upper surface		Lower surface	
				Total	Positive	Total	Positive
1	Paraffin wax	1	2–8	4	3	5	4
2	Sira wax	1	2–8	3	Nil	4	2
3	,,	5	1	5	3	5	3
4	,,	5	1	5	Nil	5	3

Complete series of microtome sections of the portions of leaves exposed in certain of the trials in Exp. 4 of Table III showed many puncture traces branching freely between the membrane and the leaf, the majority penetrating only the epidermis and superficial mesophyll cells (fig. 9, Pl. 29). The sections from two successful trials from the lower surface, however, showed three and seven punctures respectively that penetrated certainly into the phloem, e.g. fig. 10, Pl. 29. The sections from two unsuccessful trials from the upper surface showed several large branching traces, e.g. fig. 11, Pl. 29; one possibly entered the xylem but none reached the phloem. On the other hand, for Exp. 3, where a 100μ membrane failed to prevent infection from the upper surface, histological preparations from a successful upper-surface exposure revealed two large branching puncture traces penetrating the xylem and certainly entering the phloem. In this experiment the 100μ membrane had therefore failed to give the desired control of penetration.

(4) *The effect of puncture into the coleoptile.* Infection was rarely obtained by exposing the coleoptiles of young seedlings to infective insects, whereas once the young leaves had burst through the coleoptile the plant was readily infected (Table IV). Of three experiments, one was performed by caging infective insects with seedlings recently germinated over water and

removing them before the leaves emerged from the coleoptile (so that the insects could have fed on the roots as well as the coleoptile); one by placing tubes over the coleoptiles, as they emerged from the soil; and the third by using cages made of two disks of celluloid, held 5 mm. apart by a ring of cork. These disks had concentric holes, which allowed the cage to be slipped over the coleoptile or the emerging leaves as required, exposing a 5 mm. section of either to the contained insects.

Table IV. The exposure of the coleoptiles of young maize seedlings to puncture by infective *Cicadulina mbila*

			Experimental trials			
			Contact with coleoptile		Contact with young leaves	
Exp. no.	Method of experiment	No. of insects in each trial	Total	Positive	Total	Positive
1	Germinated in test-tube. Whole plant exposed	5	10	1	4	4
2	Tube over emerging shoot	5	9	1	32	21
3	Celluloid cage around shoot	5	4	Nil	4	4

Histological preparations revealed many puncture traces into the coleoptile tissue, often of exceptional length; but owing to the deep-seated position of the two vascular bundles, no puncture could have reached the phloem (fig. 12, Pl. 29). At its line of future splitting, however, the coleoptile is thin, and it is possible—although not demonstrated—that puncture here might reach the underlying leaf. The two successful experiments in which the coleoptile alone was exposed may have been due to puncture in this way. In Exp. 3, where the insects were confined to the thick part of the coleoptile, no infections occurred.

Although the lack of infections through coleoptile tissue might be due to some particular character of the tissue itself, the most probable explanation is the inaccessibility of the phloem.

(5) *The influence of the duration of puncture.* Experiments were performed, in which the contact of infective insects was interrupted at set time-intervals; by using large numbers of insects and allowing repeated contacts, each experimental plant was caused to receive many punctures, but none exceeding in duration the chosen time.

In my first experiments ten insects were confined in a glass tube of 20 mm. diameter with its lower end covered with fine mosquito net or a thin membrane ("Baudruche Capping Skin"). This end was pressed against the leaf of the experimental plant, except when, at the required time intervals, an electrically driven cam device raised the tube clear of the leaf. Experiments allowing a succession of contacts of 15, 30 or 75 sec., totalling 12 hr., at laboratory temperatures (23–26° C.), failed to give any infections. In all, seventeen experiments were performed. The control experiments, in which the conditions were identical except that contact was not interrupted, were positive without exception.

Further series of experiments were performed with the number of insects increased to fifty and the total exposure period reduced to 1 hr. The diaphragm between insect and leaf was dispensed with, and contact was interrupted by one of the following devices: the insects were sucked from the leaf surface by a pipette, flexibly mounted in the tube, and released at once (series 1 and 2); or, the tube, equipped with a single fine wire across its lower end, was rotated one half turn, so that the wire passed over the whole of the exposed leaf surface and disturbed every insect in contact (series 3).

The results of these experiments appear in Table V. In the first series, 1 min. contacts gave no infections in six experiments; 5 and 10 min. contacts gave a small proportion of infections; while 15 and 20 min. contacts were fully effective. These experiments were performed at irregular times as opportunity allowed; but to eliminate any possible error from this cause, series 2 and 3 followed a Latin square arrangement in time. On one day one experimental test of every contact period was performed, and in such an order on successive days that each contact period occupied once the first, second, etc., place. In both series, 2·5 min. contacts gave no infections; 5 min. contacts only two infections in ten trials; and longer contacts an increasing proportion of infections. The mean temperature during the contact periods appear in column (3) of Table V.

The significance of these results is evident, particularly if the figures of columns (4) and (5) be studied. The total number of punctures in column (4) was estimated from counts of the number of insects in contact with the leaf at the end of each contact period. The approximate accuracy of the estimate was confirmed by sectioning a sample of one exposed leaf. Here the number of punctures estimated from counting the insects was 160 and from histological examination of the sample 190. Observation showed that the majority of the insects had settled on the leaf immediately after being disturbed at the beginning of the contact period, but some only reached the leaf during the course of this period; so that the figures somewhat exaggerate the number of punctures of the full nominal duration.

Table V. Infections caused by a succession of short contacts by infective *Cicadulina mbila*. Total duration of contacts, 1 hr.

(1) Series no.	(2) Individual contact periods min.	(3) Mean temperature during contact periods ° C.	(4) Mean total no. of punctures per experiment	(5) No. of insects giving equivalent continuous contact	Experimental trials	
					(6) Total	(7) Positive
1	1	23–26	280	4·7	6	Nil
	5		114	9·5	13	1
	10		45	7·5	17	4
	15		55	13·7	4	4
	20		42	14·0	4	4
2	2·5	25·5	355	14·8	6	Nil
	5		181	15·1	6	1
	7·5		134	16·7	6	2
	10		120	20·0	6	6
	12·5		89	14·8	6	4
	15		88	22·0	6	6
3	2·5	22·5	250	10·4	4	Nil
	5		143	11·9	4	1
	7·5		130	16·2	4	2
	10		100	16·7	4	2
Control	Continuous		—	1	35	12

Column (5), calculated from (4), shows the equivalent numbers of insects that would have maintained continuous contact, if they had not in fact been disturbed. By the method of estimation, the figures are inevitably somewhat too high, but to halve them would be to make ample allowance for error. Evidently, from the results already recorded, continuous 1 hr. contacts by the number of insects given in column (5) would rarely fail to cause infection. For series 3, figures for continuous contact of single insects under the conditions then ruling provide an estimate for q. Assuming independence (p. 460), a group of eight insects should fail less than four times in 100 trials ($q^8 = 0{\cdot}035$); but the results show that the equivalent contact of about sixteen insects, by 7·5 and 10 min. intervals, failed twice in four trials.

This work thus demonstrates that for infection to result contact must be maintained for a threshold period, which is close to 5 min.; and that even 10 min. contacts are rarely successful. At first sight it might be thought that the threshold period is the time necessarily occupied by the insect in inserting its stylets as far as the phloem. One-minute punctures have never been found to penetrate to the phloem. But of seventeen 2·5 min. puncture traces examined histologically four certainly had reached the phloem. About half the traces resulting from unsuccessful 5 and 10 min. contacts had reached the phloem, e.g. fig. 8, plate 29. It seems certain that failure to penetrate the phloem does not alone explain the failure of short contacts to cause infection.

I know of no other work with which these experiments are exactly comparable. There is some incidental evidence, but it leaves in doubt whether other jassid vectors require a similar threshold period for infection. Thus Bennett (1934) obtained a few infections from single 5 min. contacts of single *Eutettix tenellus* at 15·5° C., but not from 2 and 3 min. contacts; if the temperature was raised to 29° C. or more, 2 min. contacts were sometimes successful. Comparison with aphid vectors is probably irrelevant, for recent work (e.g. Watson 1936) makes it probable that the transmission process in many aphids is of a different kind from that in jassids.

DISCUSSION*

Although on some points these studies are inevitably indecisive, it is worth while to follow whither their tentative conclusions lead us.

I have shown that of several possible hypotheses the independence hypothesis best conforms with the experimental evidence. I have hitherto considered only its bearing upon the *contact* of the insects; we may now consider whether the hypothesis must not logically be extended to the *puncture*. A contact, as the term is here used, means a single puncture maintained for the whole period, or several punctures aggregating in duration to the same period. If we deny the hypothesis that the effects of punctures are independent, we must agree that two punctures by one insect can exert a combined effect, while two similar punctures by different insects cannot do so. Is it conceivable that this fundamental principle of the combinability of effects can be decided simply by the identity of the insects concerned? It is true that one may argue that one puncture by an insect may "condition" the plant to a receptive state for inoculation by that one particular insect, but by no other; or that a preliminary feed on the plant may "condition" the insect to that particular plant so that at its next puncture it is capable of inoculating that plant, but no other. Such an extremely specific interaction is hardly believable. It is also conceivable that in the insect the virus undergoes some specific change so that two inoculations by the one insect are "compatible" and can combine, whereas inoculations by different insects are "incompatible". But, apart

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* This section received in revised form 3 April 1938.

the plant can mobilize a defensive reaction in the punctured phloem cell and in no other; or that the virus, once established in the punctured phloem cell, becomes protected against further inactivation.

The alternative site of inactivation is the terminal point of delivery of the virus—the meristematic tissue of the growing point. This is the likely site, for it is only here that the virus begins to exert a recognizable effect on the plant's development. On this conjecture, a succession of insufficient doses would travel down the phloem to be delivered to the meristem, where they would accumulate until an infective concentration was attained. But it is difficult to believe that over such a long distance of travel to a relatively small terminal point, the succession of small doses inoculated by one insect could remain distinct from those inoculated by other insects into the same leaf. Particularly is this so because there are only a limited number of channels of travel, the main vascular bundles—less than 100 in the leaves here used, of which only a part were available as a direct route for the virus. Thus doses inoculated by different insects would sometimes arrive at the same terminal point with the identity of their origins lost. And yet, if the independence hypothesis be true, the doses cannot combine unless their origins are identical.

The cumulative hypothesis of inoculation thus encounters difficulties. The alternative is that inoculation is in distinct doses, each one independent of any others. Such a dose may be sufficient to cause infection; or, if we agree that a dose can be insufficient, then the insufficient dose is at once inactivated by the plant's defence. We need not postulate where the defensive reaction occurs. The time of delivery of a dose of virus cannot be determined by factors independent of the insect's behaviour in puncturing the leaf, for otherwise subthreshold punctures would sometimes succeed. But it seems entirely reasonable to suggest that some incident, that occurs only after puncture has been maintained for more than the threshold period, may be the critical feature exciting the delivery of the dose. When the mouthparts are inserted, the insect on the one hand begins to receive a flow of food solution which by its mere bulk or by its nutritive effect may stimulate a reaction; the plant, on the other hand, is suffering a withdrawal of the contents of its phloem, with a possibly profound effect on its local physiology. After some time the insect ceases to suck and the original conditions may be in part or entirely restored. There is thus ample opportunity for a time factor to operate.

My argument thus leads me to extend the independence hypothesis even to the dose of virus and to reject the mass action hypothesis of Carsner and Lackey (1929), in so far as it postulates that the effective size of the dose can be determined by the accumulation of separate doses inoculated in different places or at different times.

Carsner and Lackey based their opinion upon studies of the curly-top virus of sugar beet and its vector, the leaf-hopper, *Eutettix tenellus*. This also was the material of Bennett's work, with which I frequently compare my own. We must note one striking point of difference between this and the maize streak virus. I agree with Bennett (1934) in finding that the virus must be directly inoculated into the phloem if infection is to follow. But whereas the curly-top virus remains almost completely confined to the phloem (Bennett 1936) throughout its existence in the plant, the streak virus enters the mesophyll tissue and is thence taken up by the insect. This difference between the two viruses is in conformity with the effects that they produce in the plants; the one mainly a generalized reduction in growth and development, together with phloem necrosis, and the other a marked suppression of development in localized groups of mesophyll cells.

I acknowledge the assistance given by Mr R. F. W. Nichols in the manipulation of the experiments here described; and the advice of Mr F. Yates in the statistical interpretation of several of them.

SUMMARY

Experimental studies in transmitting the maize streak virus by *active* races of *Cicadulina mbila* (i.e. always able to transmit if allowed prolonged contacts with the plants) led to the following conclusions:

(1) The probability of infection following a short contact (e.g. 1 hr.) depends in part on a variable inherent quality in the insect, termed its *potency*.

(2) The effect produced by one insect (on the most reasonable interpretation of the evidence) is independent of the effects of any others; and the probability of infection by a group is the probability that one or more of its members would alone have infected.

(3) The maize plants used varied somewhat in susceptibility.

(4) The probability of infection is not considerably influenced by the removal of the inoculated leaf tissue shortly after the inoculation; nor is it influenced by the length of leaf through which the virus must pass in moving to the stem.

(5) The insect can take up virus from the mesophyll in a chlorotic area of a diseased leaf, but cannot take it up from either the mesophyll or phloem of a green area.

(6) An insect can sometimes infect during a single maintained puncture, provided that it penetrates to the phloem. Infection fails if the punctures are artificially prevented from reaching the phloem.

(7) Punctures, if prolonged for less than a threshold period, near to 5 min., always fail to infect.

A consideration of possible alternatives causes me to favour the hypothesis that the insect inoculates the virus in distinct doses, each independent in its effect of any other doses that may be inoculated by the same or other insects; and that the delivery of a dose is determined by some incident that occurs only after puncture has been maintained for some time.

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EXPLANATION OF PLATES

Photomicrographs of sections of maize leaves, stained with gentian violet and orange G, showing the traces of punctures of *Cicadulina mbila*. Cut in longitudinal plane, unless otherwise stated. All photographs are orientated with the upper (adaxial) surface of the leaf at the top.

Plate 28

FIGS. 1–4. Successive sections, showing one branch of the trace of a successful puncture of 59 minutes' duration. The trace branches in the mesophyll (fig. 1); passes through the bundle sheath (fig. 2); enters the phloem (fig. 3), and there ends (fig. 4), with a large but ill-defined sheath and a mass of the sheath material passing down one phloem cell. × 545.

FIG. 5. The termination of the trace of a successful puncture, from the upper surface, of 60 min. duration. After passing through the xylem (sheath showing out of focus) the trace ends in a phloem cell adjacent to the xylem. × 545.

FIG. 6. The trace of one of several punctures made during a successful 1 hr. contact. The trace passes from the lower surface of the leaf straight to the phloem. The termination of the sheath is made of orange-staining material. × 545.

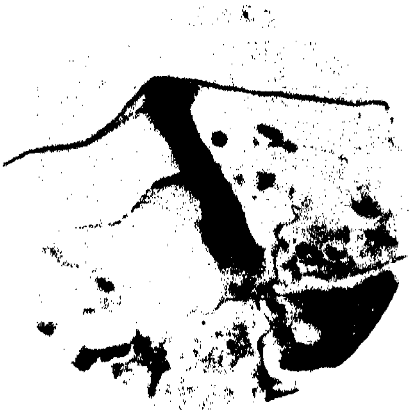

Fig. 1

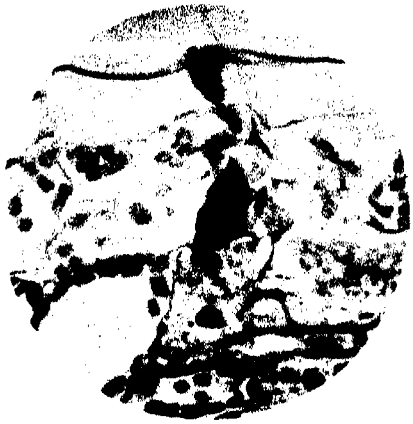

Fig. 2

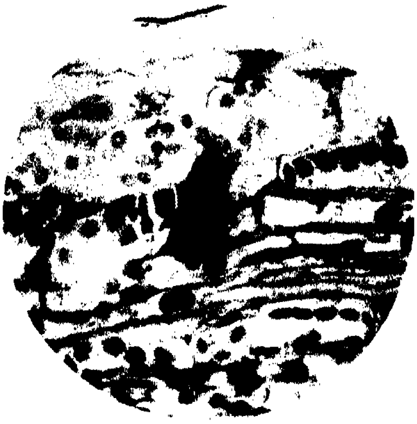

Fig. 3

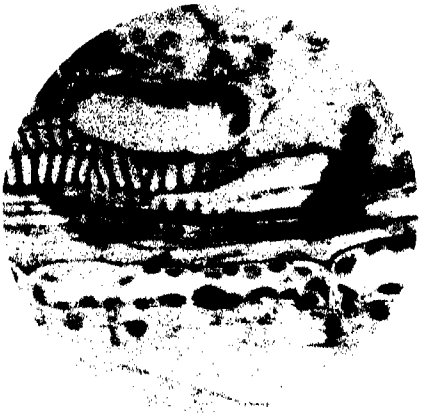

Fig. 4

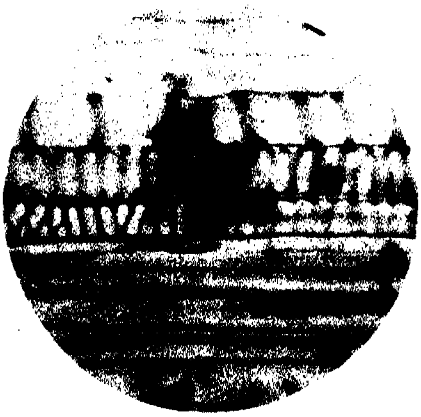

Fig. 5

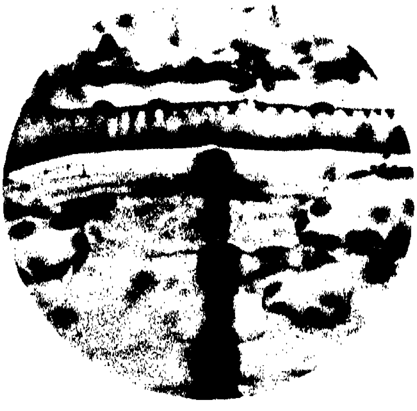

Fig. 6

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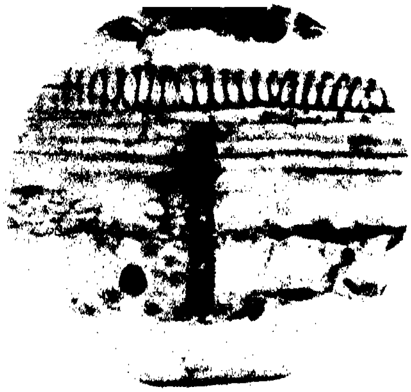

FIG. 7

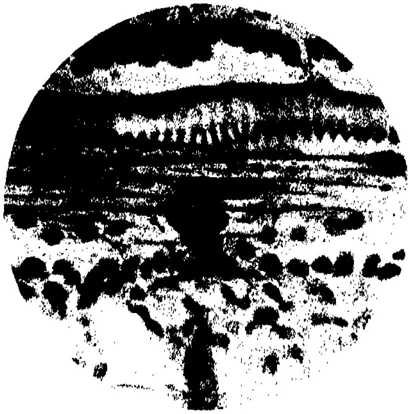

FIG. 8

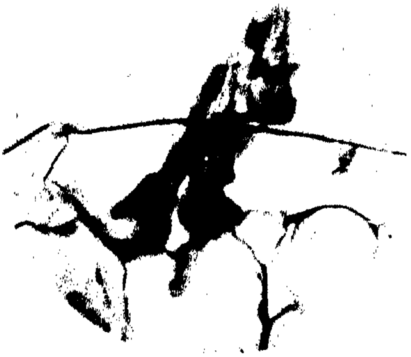

FIG. 9

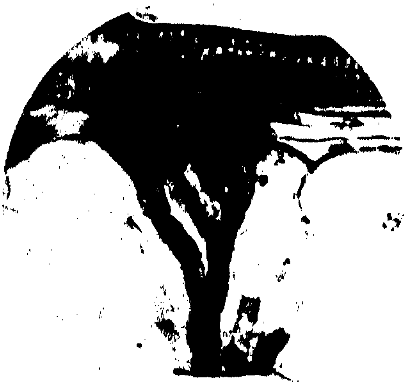

FIG. 10

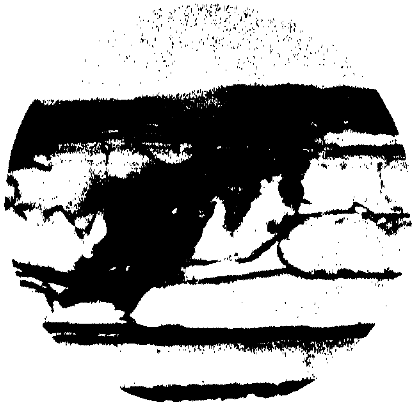

FIG. 11

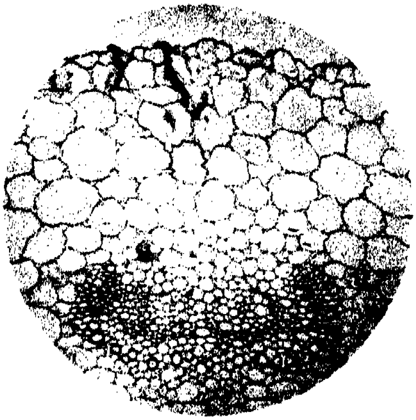

FIG. 12

Plate 29

FIG. 7. The trace of an unsuccessful puncture of 45 min. duration. The sheath ends in the phloem. × 545.

FIG. 8. The termination in the phloem of the trace of an unsuccessful puncture of 10 min. duration. × 545.

FIGS. 9–11. The traces of punctures made through a wax membrane, 100μ thick, into maize leaves held in contact with the membrane. From experiments of Table III. × 490.

FIG. 9. Trace typical of the majority of punctures, branching freely between the membrane and the surface of the leaf, and some branches entering the epidermal and mesophyll cells.

FIG. 10. Trace of one puncture from a successful exposure of the lower surface, in Exp. No. 4. The much-branched trace penetrates the phloem extensively and enters the xylem.

FIG. 11. Part of the trace of one puncture from an unsuccessful exposure of the upper surface in Exp. No. 4. An extensive branched trace penetrates to the bundle sheath but fails to reach the phloem.

FIG. 12. The coleoptile of a maize seedling, in transverse section, showing one of the two vascular bundles and a puncture trace, which, although of unusual length, fails to approach the phloem. × 105.

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The control of beating and of micro-fibrillation by means of potassium, calcium and sodium in the chick embryo heart

Potassium fibrillation

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Introduction

In a previous paper (Murray 1938) it was shown that fibrillation by excess of calcium in the medium depended upon an abnormally high ratio between calcium at the cell surfaces and potassium in the cell interiors. In the present paper further experiments are described, from which it will be concluded that fibrillation by excess potassium in the medium depends upon a similar relation, but in this case between potassium at the cell surfaces and potassium, and perhaps also sodium, in the cell interiors.

The methods used were the same as in the experiments described earlier (Murray 1938). The media were pure salines, or saline-glucose, in watch glasses. Table I gives the compositions of the various solutions. In the text, an arrow between the names of two solutions means that hearts were first treated in the solution on the left of the arrow and then transferred to the solution on the right. For further details, refer to the earlier paper.

Part I

It is first necessary to describe experiments which show that potassium fibrillation cannot occur, or can only occur weakly, in hearts which are calcium-poor, a fact which added greatly to the difficulty of investigating potassium fibrillation.

Exp. 1

PC ↘		5 hearts, pure salines
$NaCl + CaCl_2$ ↘	Fibrillating solution 1	6 ,, ,,
$NaCl + KCl$ ↗	without calcium	6 ,, ,,
$NaCl$ ↗		6 ,, ,,

Object. To examine the ability of hearts to fibrillate in large concentrations of potassium after previous treatment in solutions containing sodium, potassium and calcium (*PC*), or lacking either potassium or calcium ($NaCl + CaCl_2$ and $NaCl + KCl$), or lacking both potassium and calcium (NaCl).

Hearts previously treated with either *PC* or $NaCl + CaCl_2$ showed active fibrillation in fibrillating solution 1 without calcium, but after treatment with $NaCl + KCl$ or NaCl the fibrillation was very much weaker, usually extremely weak or absent.

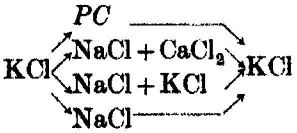
TABLE I. COMPOSITION OF SOLUTIONS g. %

Symbol of solution	NaCl	KCl	$CaCl_2$	Remarks
PC	0·68 or 0·75	0·06	0·03	NaCl was 0·75 % or 0·68 % in different experiments. The difference had no detectable effect
$NaCl + CaCl_2$	0·68 or 0·75	—	0·03	Ditto
$NaCl + KCl$	0·68 or 0·75	0·06	—	Ditto
NaCl	0·75	—	—	
KCl	—	0·96	—	
Paralysing solution 3	0·75	0·4	0·03	
Ditto, without calcium	0·75	0·4	—	
Fibrillating solution 1	0·25 or 0·23	0·66	0·01	NaCl was 0·25 % or 0·23 % in different experiments. The difference had no detectable effect
Ditto, without calcium	0·25 or 0·23	0·66	—	Ditto
Ditto, glucose control	0·23	0·02	0·01	*Glucose*: 3·08
Fibrillating solution 2	0·25	0·64	—	
	Phosphate buffer			

Thus fibrillation in the presence of a high concentration of potassium was very much more active if the first solution contained calcium than if it did not.

The duration of treatment in the first solutions was 1–2 hr.

Exp. 2



6 hearts, pure salines
14 ,, ,,
6 ,, ,,
13 ,, ,,

When hearts are put into KCl they fibrillate actively, but after an hour or so the fibrillation becomes very weak. The object of the experiment was to see whether the ability to fibrillate actively in KCl could be restored by treatment with any of the four solutions used in the preceding experiment.

The hearts were all treated in the first bath of KCl until fibrillation had nearly died away and were then transferred to one of the four solutions in the middle column above. They were then left there for about 35 min., by which time fibrillation had stopped and beating returned in many of the hearts in *PC*, $NaCl + CaCl_2$, and NaCl (beating did not return in $NaCl + KCl$). They were then again placed in KCl, and it was found that fibrillation was decidedly more active in the hearts which had been treated with *PC* or $NaCl + CaCl_2$ than in those which had been treated with $NaCl + KCl$ or with NaCl. Thus provision of calcium in the second bath restored to the hearts the ability, which they had previously lost, of fibrillating actively in pure KCl.

The above experiments show that potassium fibrillation cannot occur, except very weakly, if the hearts have suffered previous treatment with solutions lacking calcium, and if the solution intended to produce the fibrillation also lacks calcium. For hearts to display potassium fibrillation they must be adequately supplied with calcium.

An important consequence of this was that in subsequent experiments, when it was desired to study the effect of potassium in producing fibrillation in potassium-poor hearts, the condition of potassium poverty had to be produced by treatment with $NaCl + CaCl_2$ and not with NaCl. The further difficulty resulting from this is described in Part II, § 3.

PART II. THE RELATION BETWEEN EXTERNAL AND INTERNAL POTASSIUM

The following facts suggested that potassium fibrillation was more readily produced in potassium-poor hearts than in hearts having the normal potassium content:

(1) The phenomenon of "double fibrillation". When hearts treated with NaCl are transferred to solutions containing both potassium and calcium there occurs a transient fibrillation which ceases after a few minutes. The previous paper showed that this was caused by the calcium in the second solution, but it was mentioned that the transient fibrillation sometimes showed two peaks of activity, separated by an interval of comparative quiescence. This double fibrillation was difficult to recognize except when the quiescent phase was very marked so that the peaks of activity were well differentiated; for this reason it probably occurred much more often

than I was aware. The occurrence of two outbursts of fibrillation suggested the action of two agencies producing fibrillation at different intervals after immersion of the heart in the second solution, and the only change made was the addition of potassium and calcium. That potassium was responsible for the second peak of activity was suggested by the absence of double fibrillation when the solution did not contain potassium, but since under these circumstances the fibrillation was not transient it was possible that the second peak was masked. If the second peak is to be attributed to potassium its transient nature was of course due to the potassium-poor cells taking up potassium from the medium just as in transient calcium fibrillation (Murray 1938).

(2) In a repetition of part of Exp. 2, fourteen hearts were left in KCl for 55 min., by which time fibrillation was either very weak or absent. Seven of them were then transferred to NaCl + KCl and seven to NaCl for half an hour, and then all were again placed in KCl. Fibrillation in the second KCl was in all weaker than in the first, as expected from Exp. 2, but was definitely more active in the group treated with NaCl than in that treated with NaCl + KCl. Thus the hearts which had lost more potassium fibrillated more actively in excess potassium than those which had lost less. Since, however, fibrillation was in both groups very weak, the observation did not seem conclusive.

(3) When hearts were transferred from NaCl + $CaCl_2$ to *PC* there was transient fibrillation in *PC*. Since the only difference between these two solutions was the presence of potassium in *PC*, it would appear that the fibrillation must have been caused by this potassium. If it were possible to accept this conclusion without question, little further experimentation would be required, because it would have been shown that potassium-poor hearts (i.e. hearts treated with NaCl + $CaCl_2$, see Watchorn and Murray 1938) are caused to fibrillate by a concentration of potassium in the medium which does not have this effect on normal hearts. But unfortunately the conclusion cannot be accepted without question. NaCl + $CaCl_2$ is a solution in which fibrillation gradually develops, and this fibrillation often appears before beating stops. Furthermore, fibrillation may exist in a "latent" condition, especially if the frequency of beating is high, because the intervals between beats are then too short for the fibrillary contractions to appear. Thus a heart in NaCl + $CaCl_2$ may be examined and seen to be beating and not fibrillating; but if for some reason beating stops or is interrupted, fibrillation may then occur. When hearts are transferred from NaCl + $CaCl_2$ to *PC* they always stop beating immediately and fibrillation is then seen, and it is not possible to be quite

certain whether this fibrillation has been newly induced in *PC* or was already latent in $\mathrm{NaCl}+\mathrm{CaCl_2}$ and is revealed in *PC* because of the cessation of the beat. Most of the remaining experiments of this section were directed to overcoming this difficulty.

Exp. 3

$$\begin{tikzcd}[row sep=small] & \text{Paralysing solution 3} \quad \text{6 hearts, pure salines} \\ \mathrm{NaCl}+\mathrm{CaCl_2} \arrow[ur] \arrow[dr] & \\ & PC \quad \text{6 ,, ,,} \end{tikzcd}$$

Object. If the fibrillation appearing in *PC* after previous treatment with $\mathrm{NaCl}+\mathrm{CaCl_2}$ was really produced by the potassium in *PC*, it should be produced more actively by a solution containing more potassium than *PC*. Paralysing solution 3 is such a solution; at the same time it does not contain enough potassium to induce fibrillation in normal hearts. Hearts were treated with $\mathrm{NaCl}+\mathrm{CaCl_2}$ and were then transferred either to paralysing solution 3 (six hearts) or to *PC* (six hearts). If the hypothesis were correct, it was expected that fibrillation would be more active in paralysing solution 3 than in *PC*.

The experiment was performed as follows. From the hearts in $\mathrm{NaCl}+\mathrm{CaCl_2}$ two were selected which (1) had been in $\mathrm{NaCl}+\mathrm{CaCl_2}$ for equal times, (2) were as nearly as possible equally affected by the $\mathrm{NaCl}+\mathrm{CaCl_2}$, as indicated by the character of the beat and the nature of the fibrillation, if any. One of the two was then transferred either to the paralysing solution or to *PC* and the resulting fibrillation studied. The other was then transferred to the alternative solution and its fibrillation studied. The fibrillation in the two second solutions could thus be compared.

It was sometimes not possible to find two hearts equally affected by the $\mathrm{NaCl}+\mathrm{CaCl_2}$. In this case, the heart more affected was chosen for transference to *PC*, and that less affected for transference to paralysing solution 3. The reason for this procedure was that the heart more affected in $\mathrm{NaCl}+\mathrm{CaCl_2}$ was presumably the heart which had lost more potassium in $\mathrm{NaCl}+\mathrm{CaCl_2}$, and, if the hypothesis were correct, was therefore likely to fibrillate more actively in a potassium-containing solution. If the heart had been transferred to paralysing solution 3 a bias would therefore have been introduced in the direction favourable to the hypothesis; but by transferring it to *PC* the bias was in the direction unfavourable to the hypothesis.

Among the six pairs of hearts studied, in four pairs the heart transferred to paralysing solution 3 fibrillated more actively than that transferred to *PC*, although in three of these pairs there was a bias, produced in the manner just described, towards greater fibrillation in *PC*. In a fifth pair there was more fibrillation in *PC*; the bias was in this direction. In one pair the fibrillation in the two hearts was about the same.

Thus there was more fibrillation in the solution containing more potassium, in spite of the bias in the opposite direction. The result evidently favours the view that it was the potassium which was responsible for the fibrillation in both solutions.

It should be added that the osmotic differences between the three solutions were not compensated; it is, however, known (Murray 1935) that transferences from high to low osmotic pressures may produce fibrillation, if the change be great, but that the same is not true of changes in the reverse direction. In this experiment *PC* and NaCl + CaCl$_2$ have nearly the same osmotic pressures while paralysing solution 3 has a considerably higher osmotic pressure.

Exp. 4

NaCl + CaCl$_2$ ↗	Paralysing solution 3 without calcium	22 hearts, pure salines
↘	NaCl + KCl	23 ,, ,,

Object. Repetition of Exp. 3, eliminating calcium from both second solutions. The purpose of the omission of calcium was to reduce the danger of confusion between fibrillation excited by the potassium in the second solutions and any calcium fibrillation that might have survived from the first solution. In addition to this new precaution, all the precautions taken in Exp. 3 were here repeated, with one accidental exception: in one pair of hearts, that heart which was more affected in NaCl + CaCl$_2$ was transferred to paralysing solution 3 without calcium instead of to NaCl + KCl.

As in Exp. 3, if the fibrillation occurring in the potassium-containing solutions were caused by the potassium, there should be more fibrillation in the paralysing solution than in NaCl + KCl, because the former contains more potassium.

The forty-three hearts were studied in twenty-three pairs, one heart transferred to paralysing solution 3 without calcium being used as pair fellow for two hearts transferred to NaCl + KCl.

The results are summarized in Table II. When the hearts were equally affected in NaCl + CaCl$_2$ (line 2 of the table), there was in six or eight pairs more fibrillation in the hearts transferred to the paralysing solution, and in only one pair more fibrillation in the heart transferred to NaCl + KCl. When the hearts were not equally affected in NaCl + CaCl$_2$ and the bias was thrown (by the procedure described for Exp. 3) in favour of more fibrillation in NaCl + KCl (line 1 of table), there was still in a majority of cases more fibrillation in the paralysing solution. When the bias was thrown by accident, in one pair, in favour of the paralysing solution (line 3) there was more fibrillation in that solution. If the differences in the effect of $NaCl + CaCl_2$ are ignored, and the three groups of hearts are taken together (line 4), there were about three times as many pairs in which the heart in the paralysing solution fibrillated more actively than there were pairs in which the heart in $NaCl + KCl$ fibrillated more actively.

TABLE II

	No. of pairs	More fibrillation in $NaCl + KCl$	Fibrillation equal in both	More fibrillation in paralysing solution
(1) Hearts more affected in $NaCl + CaCl_2$ used for $NaCl + KCl$	12	3	4	4, + 1?
(2) Hearts equally affected in $NaCl + CaCl_2$	10	1	1	6, + 2?
(3) Hearts more affected in $NaCl + CaCl_2$ used for paralysing solution	1	0	0	1
(4) All hearts taken together	23	4	5	11, + 3?

Thus the results of Exp. 3 were confirmed under more stringent conditions and with larger numbers of hearts.

Exp. 5

$$\begin{tikzcd} NaCl + CaCl_2 \arrow[r] & NaCl \arrow[r] & \text{Fibrillating solution 2 without calcium} & \text{22 hearts, pure saline} \\ PC \arrow[urr] & & & \text{24 ,, ,,} \end{tikzcd}$$

Object. To compare the susceptibility of potassium-poor hearts to fibrillation by potassium in the solution with that of normal hearts.

This experiment differs from those preceding it in that the last solution was the same for both groups of hearts, while one of the two groups had previously been made potassium-poor by treatment with $NaCl + CaCl_2$, the other group being normal hearts which had been kept in *PC*. The hearts treated with $NaCl + CaCl_2$ were kept in that solution for times ranging from 1¾ hr. to 3¾ hr. By this time there was clearly much fibrillation and either no beat or beating confined to the auricles. After treatment with $NaCl + CaCl_2$ the hearts were transferred to NaCl for 10 or 12 min. At the end of this time the fibrillation which had been present in $NaCl + CaCl_2$ had died away. Thus, when the hearts were transferred from NaCl to the fibrillating solution there was no possibility of confusion between fibrillation excited by the potassium in that solution and fibrillation previously excited by the calcium in NaCl + $CaCl_2$. The difficulty mentioned in Part II, § 3, was thus abolished. Therefore any fibrillation seen in the fibrillating solution must have been caused by the potassium in that solution.

The hearts treated in NaCl + $CaCl_2$ differed from those kept in *PC* in being potassium-poor. Hence any difference between the two groups in the amount of fibrillation shown in the fibrillating solution must have been associated with the difference in potassium content. It could not have been due to any significantly greater supply of superficial calcium in the hearts treated with NaCl + $CaCl_2$ because the disappearance of the fibrillation in NaCl showed that this difference had been eliminated.

The hearts were studied in pairs as in Exps. 3 and 4. The results were very clear. Among the twenty-two pairs, there was in seventeen pairs definitely more fibrillation in the heart previously treated with NaCl + $CaCl_2$ than in that treated with *PC*, and the same was probably true of the remaining five pairs, but in these the difference was not sufficiently great for certainty. In no pair was the opposite result obtained.

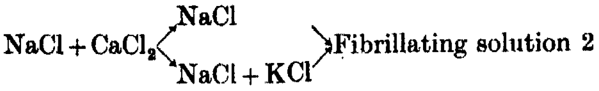
Thus fibrillation, which could only have been excited by the potassium in the solution, was more active in the potassium-poor hearts than in hearts which were not potassium-poor.

Exp. 6

$$\begin{tikzcd}[row sep=small] & \mathrm{NaCl} \arrow[r] & \text{Fibrillating solution 2} & \text{8 hearts} \\ PC \arrow[ur] \arrow[dr] & & & \\ & \text{Fibrillating solution 2} & & 8\ \text{,,} \end{tikzcd}$$

In the preceding experiment the two groups of hearts differed in two respects: the experimental group passed through both NaCl + $CaCl_2$ and NaCl, the control group through neither of these solutions. It was inadmissible to conclude that the differences in behaviour were due to the difference between NaCl + $CaCl_2$ and *PC* until it was shown that the bath in NaCl did not increase the fibrillation of the experimental group. In the present experiment all the hearts were passed from *PC* either direct to the fibrillating solution or to NaCl for 10 min. and thence to the fibrillating solution. The two groups showed no significant differences in their behaviour in the fibrillating solution, confirming the expectation that the bath in NaCl did not increase the fibrillation induced by the fibrillating solution.

Exp. 7



$$NaCl + CaCl_2 \begin{array}{l} \nearrow NaCl \searrow \\ \searrow NaCl + KCl \nearrow \end{array} \text{Fibrillating solution 2}$$

13 hearts
9 ,,

The preceding experiments show that hearts first treated with a potassium-free medium ($NaCl + CaCl_2$) are more susceptible to fibrillation by potassium than normal hearts (previously treated with *PC*). If this loss of resistance to the fibrillating action of potassium depends upon a loss of potassium from the cells, the resistance should be restored by a bath in a solution containing potassium; compare the similar experiment in the preceding paper (Murray 1938, Part VI). Twenty-two hearts were treated with $NaCl + CaCl_2$ as in the preceding experiments and of these thirteen were transferred to NaCl and nine to NaCl + KCl. After approximately 10 min. all the hearts were transferred to the fibrillating solution. In those hearts which had passed through the intermediate bath of NaCl there was more fibrillation than in those which had passed through NaCl + KCl; that is, provision of potassium in the intermediate bath at least partially restored the ability to resist the fibrillating action of potassium in the medium. Thus there is an antagonism by potassium in the intermediate bath to potassium in the third bath; potassium antagonizes itself.

Exp. 8

Fibrillating solution 1:	4 hearts
Fibrillating solution 1, glucose control:	4 ,,

The object of this experiment was to make sure that, in fibrillating solutions 1 and 2, the fibrillation seen was produced by the large quantity of potassium and was not merely an effect of the low concentration of NaCl. Four hearts were put into fibrillating solution 1 and four into the solution called in Table I "fibrillating solution 1, glucose control", in which the excess of KCl was replaced by isotonic glucose. In fibrillating solution 1 beating quickly stopped and was replaced by fibrillation throughout the heart, while in the glucose mixture all the hearts were still beating after half an hour, though in two incompletely, and there was very little fibrillation. Later, all four hearts of this group stopped beating but there was always much less fibrillation than had been present in fibrillating solution 1 from almost immediately after immersion.

PART III. THE RELATION BETWEEN POTASSIUM FIBRILLATION AND INTRACELLULAR SODIUM

The experiments of this section will be described as briefly as possible because the results, while strongly suggestive, could not be made conclusive.

It was known that sodium in the medium antagonized the production of fibrillation by potassium and calcium in the medium (1935, 1938), that calcium fibrillation was opposed by potassium within the cells, and (from the experiments of Part II) that potassium fibrillation was opposed by potassium within the cells. It therefore seemed possible that potassium fibrillation might be opposed by sodium within the cells.

This suggestion was supported by the following experiments: (1) If hearts are placed in a *PC* all of whose salts have been equally diluted to give an osmotic pressure equivalent to NaCl 0·4 % (the low osmotic pressure being compensated by glucose), beating soon stops and is succeeded by weak fibrillation. (2) If in the dilute *PC* the KCl and $CaCl_2$ are kept at the same level as in ordinary *PC*, the same result is obtained but the fibrillation is more active. (3) If $NaCl + CaCl_2$ is prepared with the NaCl diluted but the $CaCl_2$ at the ordinary level, and if the fibrillation in this solution is compared with that in *PC* with NaCl diluted but with KCl and $CaCl_2$ at the ordinary level, it is found that hearts in the potassium-free solution fibrillate less than hearts in the potassium-containing solution. This result was obtained on two occasions. On three other occasions practically no fibrillation occurred in either group, so that no comparison could be made. These three failures are not evidence against the hypothesis, but neutral; they do, however, make it impossible to put forward the hypothesis, that sodium-poor hearts are more susceptible to potassium fibrillation than normal hearts, as more than a suggestion.

PART IV. DISCUSSION

The principal facts bearing on the conditions of potassium fibrillation are: (1) large quantities of potassium in the medium excite fibrillation in normal hearts; (2) hearts which have been treated with potassium-free solutions are more susceptible to fibrillation by potassium in the medium than are normal hearts; (3) hearts treated with potassium-free solutions become potassium-poor (Watchorn and Murray); (4) hearts which are first treated with a potassium-free solution, so that they become abnormally susceptible to potassium fibrillation, and are then treated with a solution containing a small quantity of potassium, recover the normal power of resistance to the fibrillating action of potassium in the medium. In other words, potassium in the medium excites fibrillation, and does so more readily if the hearts contain less than the normal amount of potassium; potassium in some way contained in the hearts opposes the action of potassium in the medium.

A previous paper showed that the fibrillation which is excited by calcium in the medium is not opposed, and is probably assisted, by potassium in the medium, but that it is opposed by potassium within the cells.

Thus potassium in some way contained within the hearts opposes fibrillation by either potassium or calcium in the medium; and potassium in the medium itself excites fibrillation and does not oppose the induction of fibrillation by calcium.

The behaviour of potassium is therefore paradoxical, but the paradox disappears if potassium acts differently in different situations.

In the preceding paper it was shown that all the numerous experimental facts, some of which involved very complicated series of events, pointed to, and were satisfactorily interpreted by, the hypothesis that calcium fibrillation depended on a disturbance of the ratio between calcium acting at the surfaces of the cells and potassium acting within the cells. Calcium fibrillation occurs when the ratio superficial calcium/intracellular potassium rises sufficiently above the normal level. It is now suggested that potassium fibrillation depends upon a similar change in the ratio potassium at the surfaces of the cells/intracellular potassium.

In favour of this is first the fact that potassium in some way contained in the hearts acts antagonistically towards potassium in the medium, and the unavoidable inference from this that potassium has opposite actions in different situations. Secondly, the anti-fibrillating action is still exerted in hearts after treatment with a potassium-free solution. Thirdly, when a potassium-poor heart is transferred to a potassium-containing solution, there is seen first an induction of fibrillation by the potassium in the medium, so that the heart fibrillates more than a normal heart in the same solution, and then a decrease or stoppage of the fibrillation, so that finally the heart fibrillates only as much as a normal heart would do in the same solution. It is suggested that the heart fibrillates because the potassium in the medium gains access to the cell surfaces before it reaches the cell interiors, so that the ratio superficial potassium/intracellular potassium rises, and that the fibrillation decreases or stops when potassium from the medium enters the cell so that this ratio falls back to normal. Fourthly, this hypothesis brings potassium fibrillation into line with calcium fibrillation.

The alternative hypothesis, that potassium fibrillation occurs when there is a rise in the reciprocal of the above ratio, i.e. a rise of intracellular potassium/superficial potassium, is not in accord with the third and fourth considerations of the last paragraph, and is refuted by the occurrence of fibrillation in the transference $NaCl + CaCl_2 \rightarrow PC$, in which at least a part of the fibrillation is caused by the potassium in *PC*. In this solution it is clearly impossible for the intracellular potassium to rise above the normal level, and hence for the ratio intracellular potassium/superficial potassium to do so. But a rise of superficial potassium relative to intracellular potassium is expected as soon as the potassium gains access to the surfaces of the potassium-poor hearts; this rise will be transient, the ratio returning to normal as potassium enters the interiors of the cells. Such a transient disturbance of the ratio obviously accords with the transient nature of the fibrillation.

The experiments of Part III suggest that sodium-poor hearts, like potassium-poor hearts, are more susceptible to potassium fibrillation than are normal hearts, and so by analogy with the situation in regard to potassium, that intracellular sodium opposes the production of fibrillation by potassium and calcium in the medium.

Fibrillation, then, is induced by an action of calcium or potassium at the cell surfaces and is opposed by an action of potassium, and perhaps of sodium, in the cell interiors.

It is tempting to suggest a hypothesis which may take the analysis a stage further. Fibrillation can be excited by calcium, potassium, and magnesium (Murray 1935) in the medium, and weakly by lithium (unpublished), while calcium and potassium as fibrillators are known to be opposed by intracellular potassium and perhaps by intracellular sodium. All this suggests a mode of action not depending on specific properties of particular cations but rather on the properties of cations in general, that is, on their electrical positivity. Further, since the occurrence of fibrillation depends on a relation between cations at the surface and within the cells, the electrical effect is across the cell membrane, i.e. fibrillation depends upon changes in the potential gradient across the cell membrane. Since fibrillation is induced by a rise in the density of cations at the surface and is opposed by a rise in their density within the cells, it must depend upon a high positivity at the surface.

There are two difficulties, one real and one apparent, facing this hypothesis. The real difficulty is the fact that sodium is known as anti-fibrillator but not as fibrillator. This can only be surmounted by the supposition, for which I have no independent evidence, that sodium does not attach itself to the surfaces of cells as do calcium and potassium, and therefore exerts no effect at the surface except in maintaining osmotic pressure, but exerts its anti-fibrillating action, like potassium, from within the cells. This accords, of course, with its known presence inside cells. The apparent difficulty is that calcium is known as a fibrillator but not as an anti-fibrillator. This, however, agrees with the fact that muscle cells contain much less calcium than potassium or sodium, so that the effect of calcium acting internally is expected to be much smaller, and much less easy to discover, than the effect of potassium or sodium. This apparent difficulty, indeed, is really a fact favourable to the hypothesis, for it is worthy of remark that the ion, potassium, whose action as anti-fibrillator is most readily discoverable, should also be that which is present in highest concentration inside muscle cells, while calcium, which has not been recognized as an anti-fibrillator, should be present in muscles only in low concentrations. If the cells were rich in calcium and poor in potassium the failure to discover an anti-fibrillating action of calcium would be a real and serious difficulty.

The previous paper showed that beating depended on a balance between two ratios. A rise of the ratio superficial calcium/intracellular potassium, if not excessive, improves beating, while a rise of the ratio superficial potassium/intracellular potassium inhibits beating. Here it does not seem likely that a simple hypothesis of changes in the potential gradient across the membrane can be made to fit the facts but that the specific properties of the two ions must in some way play an important part.

SUMMARY

1. Hearts which have been made potassium-poor are more susceptible to fibrillation by potassium in the medium than are normal hearts.
2. Hearts which have been made potassium-poor recover the normal ability to resist the fibrillating action of potassium in the medium if they are treated with a potassium-containing solution.
3. It is concluded that potassium fibrillation occurs when the ratio superficial potassium/intracellular potassium becomes abnormally and sufficiently high.
4. The suggestion is made that fibrillation may depend upon a change in the electrical gradient across the cell membrane.

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Investigations on Mediterranean kala azar. XI
A study of leishmaniasis in Canea (Crete)

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In previous communications (Adler and Theodor 1931, 1935) the following sandflies of the *major* group were recorded from Greece: *Phlebotomus major*, *P. perniciosus* var. *tobbi*, *P. chinensis* var. *simici* and *P. perfiliewi* (syn. *P. macedonicus*). *P. perfiliewi* was at first suspected as the carrier of visceral leishmaniasis in Greece, for it was the only sandfly of the *major* group known from this country up to 1931, the other species not having been found to occur in the peninsula and islands till 1932. A rapid survey carried out during August 1932 in Athens and the vicinity of Argos indicated that the distribution of *P. major* in these parts corresponded closely to that of visceral leishmaniasis, and feeding experiments on infected dogs carried out in Catania and Sicily showed that this species is even more receptive for *Leishmania infantum* than is *P. perniciosus* (1932).

It was thought advisable to make an intensive study of a single endemic focus in Greece in order to test the conclusions made on the basis of the preliminary survey and compare the role of the various local species of sandfly of the *major* group as vectors of visceral leishmaniasis.

Canea in Crete was chosen because it is a centre of both visceral and cutaneous leishmaniasis, is easily accessible and has laboratory facilities. A preliminary survey of Crete, particularly in the neighbourhood of Candia, was carried out; villages where, according to the reports of medical officers, cases had occurred within the previous two years, were examined, and it was quickly established that *P. major* was present in all parts where visceral leishmaniasis had been proved to exist.

No part of Crete has as many records of visceral leishmaniasis as Canea, and because of increased facilities following the establishment of a government laboratory, the number of cases diagnosed has increased within recent years. During 1930 only twelve cases were identified in Canea, but between 1931 and 1934 there was a total of 160 cases (Papantonakis 1935).

One of the main characters of Mediterranean visceral leishmaniasis is its stability, and in foci which have been studied intensively over a period of years no annual fluctuation in numbers of cases has been observed. A conservative estimate of the incidence in Canea gives a figure of fifty cases per annum in a population of 30,000. The age distribution is the same as that previously recorded for Catania (1931) and is typical for the disease throughout the whole Mediterranean basin.

Whereas in other Mediterranean foci the disease is occasionally confused with chronic malaria, Cooley's disease is sufficiently common in Crete to be of practical importance in the differential diagnosis of visceral leishmaniasis. Between July and November 1934 seven patients suffering from this disease were sent to us as suspected cases of visceral leishmaniasis because they had chronic fever and large spleens and were not improved by quinine. The differential diagnosis is not difficult, for in Cooley's disease cultures from the blood and spleen punctures are negative as regards leishmania while the blood picture is typical. Six of our seven cases showed asymmetrical hyperplasia of various bones, particularly the parietals and zygomatic arch. The latter phenomenon produces a typical facies which together with the large spleen (reaching below the umbilicus in some cases), fever and anaemia, is pathognomic. Although the majority of cases die before reaching the age of 9, one of our cases was in a girl of 14 and another in a man of 24; both were typical and gave a history of fever and large spleen from earliest infancy. As our observations were limited to a part of the small population of Canea it would appear that Cooley's disease is relatively common in Crete.

The estimate of fifty cases of kala azar per annum among a population of 30,000 gives no indication of the local frequency of the disease, because it is limited in distribution to a few well-defined areas on the periphery of the town, the greater part of the population living in districts almost free from infection. Of the cases 70 % come from Hagios Joannis, a small district situated on the south-east corner of the town; the remaining 30 % are scattered round the periphery mainly on the eastern side of the town.

The remarkable restriction of the disease to well-defined areas can be judged from the following facts (see map for which we are indebted to Dr E. Papantonakis). The distance between the most easterly and westerly portions of the town is 2·25 km., and that between the farthest northern and southern parts is 1·2 km. The district of Hagios Joannis measures only about 300 by 250 m. and lies about 1 km. from the old Turkish city which is practically free from visceral leishmaniasis. One small endemic focus (Kumkapi) with a record of seven cases is only 350 m.

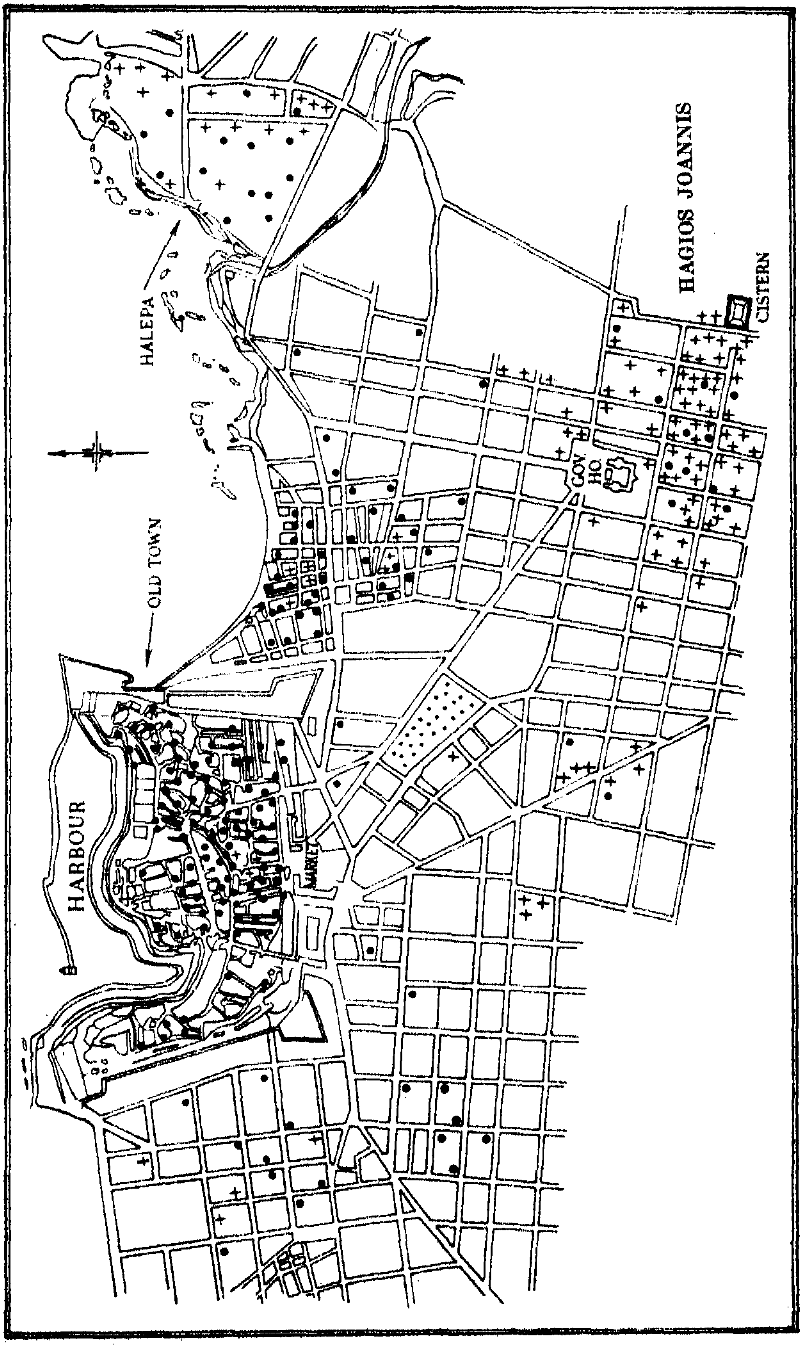

FIG. 1. Plan of Canea.

from the old city. There is no very marked difference between the economic and social status of endemic and non-endemic centres. The endemic foci are of more recent construction than the parts free from the disease, while the sanitary conditions are distinctly superior since the endemic foci contain the new residential quarters. Hagios Joannis was constructed during the last 18 years. The most notable difference between the infected and non-infected parts of the town is the presence of gardens and vegetation in the former. The almost clear-cut division into endemic and non-endemic centres within a very limited area is more striking in Canea than in any part of the Mediterranean so far investigated, and this fact has facilitated the detection of the insect vector, i.e. *P. major*.

SANDFLIES OF CRETE

Observations on sandflies in Canea were carried out between the beginning of July and 20 November 1934. During this period a total of about 7000 female sandflies was collected.

The following species were found: *P. papatasii*, *P. major*, *P. chinensis* var. *simici*, *P. sergenti*, *P. parroti* var. *italicus*. (A single male of *P. laroussei* (= *P. vesuvianus*) was found in Serakino, which will be described below.)

It soon became evident that the large majority of sandflies collected in houses were *P. papatasii*, while those collected on the walls outside houses were *P. major*, *P. chinensis* var. *simici* and *P. sergenti*. As *P. papatasii* was soon found to play a negligible role in the propagation of both cutaneous and visceral leishmaniasis most of the collecting was done out of doors.

As the distribution of human and canine visceral leishmaniasis and of cutaneous leishmaniasis was known accurately, it was easy to correlate the distribution of species of sandfly with that of these diseases street by street. The old city, which is mediaeval in construction, with narrow cobbled streets and without gardens, was carefully examined because, though free from visceral leishmaniasis, it is an intense focus of cutaneous leishmaniasis. In this area *P. sergenti* and *P. papatasii* were common (about 60 % of all the males caught were *P. sergenti*), but only five specimens of *P. major* were taken throughout the whole season and not a single specimen of *P. chinensis* var. *simici*. This observation narrowed the issue of determining the vectors of cutaneous and visceral leishmaniasis to *P. sergenti* and *P. papatasii* for the former and to *P. major* and *P. chinensis* var. *simici* for the latter. In Crete *P. parroti* var. *italicus* feeds mainly on geckoes and never on man.

Hagios Joannis, the most important centre of visceral leishmaniasis, was examined street by street, and all the five species of sandflies were found. As regards out-of-door collecting, *P. sergenti* was the commonest species in the first half of July. During August and the early part of September there was a considerable fall in the number of sandflies caught, and *P. major* predominated. After the middle of September the number increased and reached its peak in the first half of October. During this period 90 % of all sandflies caught out of doors were *P. major*. From the end of October to 20 November the only species was *P. major*. It is important to note that neither *P. perfiliewi* nor *P. perniciosus* var. *tobbi* were found during the whole period of observation. *P. chinensis* var. *simici* was not common at any time.

During the whole period of our observations in Canea the collection of sandflies was carried out by the same workers in one area. They collected under uniform conditions during several hours every evening commencing an hour or so after dusk. On this account it is possible to express the relative frequency of *P. major* at various times:

	Approximate no. of females *P. major* caught per evening
Beginning of July to the end of August	40–50
First half of September	20
Second half of September	30–40
First half of October	80–120
Second half of October	50–70
First half of November	20–30

The last generation of *P. major* appears from the second half of September to the middle of October, and its number declines rapidly in November.

The population of *P. major* in the endemic foci of Canea is not as dense as that of *P. perniciosus* in Catania or Malta, and over the period covered by our observations at least four times as many specimens of *P. perniciosus* were collected in previous years in Catania or Malta by the same workers who collected *P. major* in Canea.

Our studies have indicated very clearly that in Canea the sandfly *P. major*, and both canine and human visceral leishmaniasis are always associated and have an identical distribution.

Bionomics of P. major *in Canea.* Although this sandfly is referred to as *P. major* throughout this paper, bionomically it is very different from *P. major* as studied in previous years in Catania. In colour it is darker; the unfed females are not so markedly phototropic; it lays larger batches of eggs. Whereas *P. major* in Catania lays from seven to twenty-one eggs, in Canea the number of eggs at a single laying varied from fourteen to eighty-four. Moreover, the Canea sandfly was found to be rather variable in its external characters, particularly in October when it is most abundant. Thus a few individuals were found with rather thick torpedo-shaped abdomens in contrast to the long and slender abdomen of *P. major* in Catania, and a number of specimens appeared to resemble *P. major* var. *syriacus* rather than *P. major* itself. (Both these sandflies have been considered in detail in previous communications.) In their anatomical details some specimens were indistinguishable from *P. major* except in colour, although, as emphasized above, their bionomics were quite different. They were kept in the laboratory in an apparatus identical with that used previously for *P. perniciosus* (1935), i.e. a small cylindrical glass tube kept at a relative humidity of 100 %. A small improvement was introduced which enabled the sandflies to be maintained in the laboratory for as long as 24 days without damage to their wings and legs. The inner surface of the tube was lined with blotting paper except for a narrow window to permit observation (Fig. 2). This is by far the most satisfactory method we have tested for maintaining in the laboratory Mediterranean sandflies of the *major* group. It has also proved very satisfactory for *P. papatasii* and gives the maximum of survivals after egg laying.

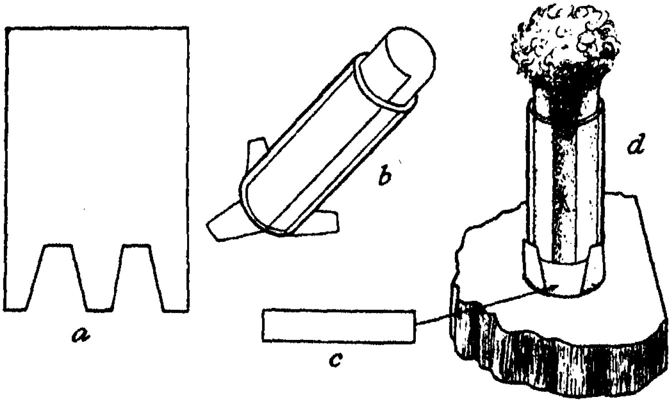

Fig. 2. Chamber for keeping sandflies in laboratory: *a*, shape of filter paper to be inserted into the glass cylinder; *b*, glass cylinder with inserted filter paper; *c*, piece of filter paper to close the lower part of the slit between the ridges of *a*; *d*, apparatus fixed in porous stone.

It must be admitted, however, that the above conditions do not correspond to natural ones, with the result that important differences were found to exist between the bionomics of *P. major* in nature and in the laboratory. In the first place very few specimens either of wild females or laboratory-bred ones copulated in captivity, no matter how the conditions were varied as regards light, temperature and humidity. For this reason it was difficult to raise successive generations of laboratory-bred sandflies, and most of our laboratory-bred specimens were obtained from eggs laid by wild females, caught after they had gorged themselves with blood. In the laboratory the highest degree of longevity in both males and females was obtained in sandflies kept at a constant relative humidity of 100 %. In nature the females do not remain constantly in an environment of 100 % relative humidity as was shown by the following experiment.

A sandfly trap was arranged in a district where *P. major* predominated. This consisted of a small room about 1 m. wide, 2 m. long, and 1½ m. high. It was provided with one sandfly-proof door but was devoid of windows. In one corner of the room a small heap of stones was placed in earth, kept constantly moist by porous jars containing water, so that the relative humidity between the stones and the porous jars was about 100 %, while the relative humidity in the room beyond the heap of stones varied from 60 to 80 %. The door was left open during the night and was closed in the morning. Recently gorged females were always found between the stones and porous jars in the morning, i.e. at a relative humidity of 100 %. It was found that during the day individual females left the environment of 100 % relative humidity and rested for varying intervals on the walls in an atmosphere of 60–80 % relative humidity, after which they returned to the resting places between the stones. It would appear that in nature the gorged female of *P. major* seeks an environment of 100 % relative humidity, but that during digestion of the blood meal it changes this environment from time to time and remains for short periods in an atmosphere of lower relative humidity according to undetermined physiological needs.

Other significant differences between *P. major* under laboratory conditions and in nature were found, particularly in the disposal of the blood meal. Females with an empty alimentary tract which had already laid a batch of eggs could be distinguished from newly hatched and unfed ones by the presence of granules in the accessory glands of the oviduct. These granules are absent in unfed primiparous sandflies. In the laboratory, females, after laying a batch of eggs, always had debris of a former blood meal in the stomach or hindgut, whereas the midgut and hindgut of wild sandflies were always free from all traces of a blood meal after egg laying. Almost all the captured females of *P. major* were completely empty and revealed no traces of a blood meal, while a minority only were recently gorged. Very few females of this species with a partially digested blood meal were caught in the evening, an indication that in the wild state the sandfly does not refeed prior to egg laying, whereas in the laboratory it refed once before egg laying in the case of fertilized females (in the relatively few instances in which these were available), and up to five times in the case of unfertilized females. (As in the case of *P. perniciosus* the eggs of fed unfertilized females could readily be distinguished from those of fertilized ones because the former do not proceed beyond a certain stage of development.) In all cases in the laboratory the females which refed still had remains of a former blood meal in the stomach. A number of females had difficulty in defaecating in the laboratory, and the insects were in distress because of large syrupy masses of faeces which remained adherent to the anus and from which they could not free themselves. When these masses were dissected off with a fine needle the insect recovered. In about 1 % of females in the laboratory a further abnormality not found in wild insects was observed. It consisted in the presence of haemoglobin crystals in the stomach. Sandflies showing this condition after feeding on leishmania-infected dogs or hamsters were found to be negative as regards developmental forms of leishmania or to contain very few flagellates confined to the stomach.

The development of *P. major* from eggs laid in the laboratory showed no significant differences from that previously described for *P. papatasii* and *P. perniciosus*. It is important to note that hibernation of the fourth-stage larvae was not found to occur independently of temperature even in the case of larvae hatched from eggs laid towards the end of November. Thus larvae remained in diapause when kept at a temperature of about 12–16° C., but in all cases pupated and hatched when transferred to a temperature of 27–30° C.

P. laroussei *Lang. and Nitz.* (= P. vesuvianus *A. and Th.) and* P. laroussei *var.* canaaniticus *A. and Th.* The female of *P. vesuvianus* was discovered by Adler and Theodor in 1929 in the neighbourhood of Naples and described in March 1931. In 1930 the female of *P. laroussei* was found by Langeron and Nitzulescu in a re-examination of sandfly material of the collection of the Faculty of Medicine in Paris and described in January 1931. Both sexes of *P. canaaniticus* were described from Palestine by Adler and Theodor in 1931. In the same paper *P. vesuvianus* and *P. laroussei* were considered as synonyms. This was not accepted by Langeron, who considered both species as valid on the basis of differences in the spermathecae (1932). In the same year Raynal and Le Gac recorded and described a female of *P. laroussei* from the suburbs of Marseilles. In 1936 Parrot recorded a female of *P. vesuvianus* from Crete, and in 1937 he described the male from a damaged specimen also collected in Crete.

P. vesuvianus and *P. laroussei* resemble each other very closely. Measurement of antennae, palps and wings are identical. The armature of the pharynx also seems to be identical to judge from the figures of Langeron and Nitzulescu, and Raynal and Le Gac. The only difference seems to be in the spermathecae. The spermathecae of *P. vesuvianus* are very thin-walled tubes with faint transverse striation and a small head. In general characters they resemble the spermathecae of *P. chinensis*. Since the walls are very thin, it is extremely difficult in mounted specimens to follow the ducts along their whole length. Furthermore, their appearance varies considerably in different specimens. Langeron and Nitzulescu described a similar spermatheca for *P. laroussei* except that the ducts are widened into a broad ampulla. As these authors were dealing with mounted material which had to be remounted and treated with potash, it is possible that the ampulla is an artefact due to swelling and distortion of the very thin and soft walls of the ducts brought about by the action of the potash. The figure given by Adler and Theodor for the spermathecae of *P. vesuvianus* shows a duct slightly narrower than the body of the spermatheca as in *P. chinensis*, whereas Parrot's drawing of the spermatheca of *P. vesuvianus* from Crete shows a duct as wide as the body of the spermatheca. It is in any case not justifiable to maintain as two species forms which are distinguished merely by a doubtful minor character of the spermathecal duct. Both names should therefore be considered as synonyms. The description of *P. vesuvianus* was published in March 1931 and that of *P. laroussei* in January 1931, so that the latter name has priority.

In July 1934 a single male of *P. laroussei* was caught in Serakino in Crete. It resembles *P. canaaniticus* very closely but shows a few minor differences in the genitalia.

Description of P. laroussei ♂

Size. 2·5 mm. Wings: length 2·6 mm., width 0·75 mm., wing index $\alpha/\beta = 2{\cdot}7$. Palps: formula 1. 4. (2. 3.) 5. Relative length of segments: 1–5·2–5–3·7–8. Antennae: segment 3 very long (0·44 mm.), $3 > 4+5$ $A3/E = 1{\cdot}5$. The third antennal segment reaches beyond the tip of the proboscis.

Pharynx. It resembles that of the female in the arrangement of the teeth of which there is a group appearing as scales in the middle and as wedge-like teeth, pointing towards the middle, at the side.

Genitalia (fig. 3). The distal segment of the superior clasper is markedly longer than half the length of the basal segment. The two median spines are situated slightly nearer to the proximal spine than to the distal ones. The penis resembles that of *P. major* but is much shorter and tapers gradually towards the rounded tip. The inferior clasper is by a quarter longer than the basal segment of the superior clasper.

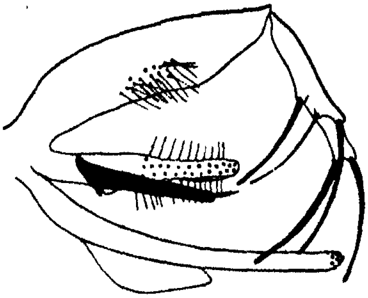

FIG. 3. *P. laroussei.* Genitalia of male.

This male resembles that of *P. canaaniticus* very closely. The only differences are (*a*) the greater length of the inferior clasper which in *P. canaaniticus* is only slightly longer than the basal segment of the superior clasper, and (*b*) the greater length of the distal segment of the superior clasper, which in *P. canaaniticus* is shorter than, or only just as long as, half the length of the basal segment.

The great length of the third antennal segment is of no significance, as the length of this segment is extremely variable. The relation $A3/E$ is equal in the specimens from Crete and in *P. canaaniticus* from Palestine. Our description of the male of *P. laroussei* corresponds on the whole to that given by Parrot except for several details. The inferior clasper is relatively longer in our specimen. The basal part of the penis differs slightly, and the genital pump has a widening at the base ("pavillon" of Parrot) as in other species of *Phlebotomus*. But as Parrot had only one much damaged specimen at his disposal these differences are possibly accidental.

P. canaaniticus was originally described from two females and four males. In the meantime other specimens were bred from a single female caught in Motzah near Jerusalem, so that more material was available for the study of this rare sandfly. *P. canaaniticus* was distinguished from *P. laroussei* mainly by the size of the teeth in the pharynx and because the termination of the spermathecae appeared to be different ("absence of a prominent projection"). An examination of seven bred females of

P. canaaniticus and the re-examination of the females of *P. laroussei* from Naples showed that the spermathecae in both species are practically identical as the size and the degree of protrusion of the knob-like projection vary to some extent. (The drawing of the spermathecae of *P. canaaniticus* may be misleading, as it seems to depict a narrow duct, whereas the ducts are actually as wide as the anterior part of the spermatheca though they are extremely difficult to see since they are just as thin-walled as in *P. laroussei.*) The appearance of the teeth in the pharynx also varies considerably according to the angle of observation, which depends on the way the specimen is mounted or on the position of the pharynx relative to the head capsule. In the case of certain females of *P. canaaniticus* the teeth were quite indistinguishable from those of *P. laroussei*. There would seem to be some justification for considering *P. canaaniticus* as a synonym of *P. laroussei*, but until it is possible to study more males of *P. laroussei* it is proposed to consider it as a variety of *P. laroussei*.

The known distribution of *P. laroussei* is therefore: France (four localities), Naples, and Crete; that of *P. laroussei* var. *canaaniticus*: Palestine.

CANINE VISCERAL LEISHMANIASIS IN CANEA

In 1933 on account of a rabies scare in the town a wholesale destruction of stray dogs commencing in May of that year was undertaken. Between July and November 1934 there were practically no stray dogs in the town, and our observations were confined to house dogs. This was actually an advantage, because the distribution of the disease in house dogs would be expected to give a satisfactory indication of the approximate sites in which the disease was acquired. Owing to the kindness of Dr E. Papantonakis, Medical Officer of Health for Canea, it was possible to examine house dogs from every part of Canea. The procedure adopted was that described in a previous paper (1932). A total of ninety dogs was examined, and of these rather more than half (fifty) came from parts of the town free from visceral leishmaniasis, while the remainder belonged to endemic foci of this infection (Hagios Joannis, etc.). *In the latter group nine animals were found to be infected and none in the former.* Actual direct contact between human cases and infected dogs could not be definitely established, for some of the human cases came from households which had no dogs, and there were houses with human cases in which the dogs were found to be free from disease. Two of the infected dogs came from houses in which human cases of kala azar occurred.

It is to be noted that no differences were observed in the ectoparasites (fleas and ticks) of dogs, in endemic and non-endemic foci, a fact which confirms the ample experimental evidence that these arthropods are not concerned in the transmission of the disease to dogs or man.

Of the nine infected dogs four showed external signs of disease, i.e. seborrhea, partial depilation, ulceration of the skin, and emaciation, and five appeared to be perfectly healthy. As observed in Malta the clinical condition, particularly the emaciation, does not necessarily bear any relation to the number of parasites to be found in the spleen. Of four animals with marked emaciation, one had few and three had numerous parasites in this organ. The heaviest spleen infection in our series was found in a perfectly healthy, well-nourished dog without any external signs of disease.

In their main outline the pathological changes found in infected animals are similar to those described in detail from infected animals in Malta (1932, 1935). There are, however, several points which call for special mention. It was found that three animals in advanced stages of emaciation barked very seldom and with difficulty—a symptom not previously recorded in this disease. Post-mortem examination showed that this was due to infiltration of the larynx and vocal cords with macrophages, small lymphocytes and plasma cells of the type previously described from other tissues.

A pathological curiosity was found in one animal, namely, a dense fibrous growth in the wall of the oesophagus containing the nematode *Spirocerca sanguinolenta*. The whole wall of this growth was infiltrated with macrophages containing numerous leishmania.

In several animals the proliferation of masses of cells indistinguishable from Kupfer cells outside the capillaries among the parenchyma cells of the liver was very striking. The majority of these cells, particularly in infected but healthy animals, were free from parasites.

A careful study was made of the cutaneous ulcers. As was to be expected, the ulcerated areas and the skin in their immediate neighbourhood contained fewer leishmania than the intact skin. This confirms the view put forward in previous papers that the cutaneous ulcers are not of epidemiological importance, and that the infection of the unbroken skin is the determining factor in the spread of the disease. The genesis of these cutaneous ulcers in canine visceral leishmaniasis is quite different from that in canine cutaneous leishmaniasis. In the latter condition ulceration follows an infiltration of the dermis with macrophages of such an intensity that it interferes with the local blood supply; in the former ulceration, usually in emaciated animals, follows degenerative changes, atrophy and necrosis of the dermis usually on sites exposed to trauma, and is not associated with particularly intense foci of cellular infiltration or parasitism.

It is important to note that none of the nine animals examined showed parasitism of the normal skin to the extent found frequently in naturally infected dogs in Malta. Sections of skin, taken from an animal at a time when about 70 % of *P. major* became infected after feeding, were compared with sections from our previously recorded series of infected dogs in Malta. Judging from the number of parasites in the skin, an infection rate of not more than 30 % would have been expected in *P. perniciosus* fed on the same animal. This confirms our previous evidence that *P. major* is a better host than *P. perniciosus* for *L. infantum*.

Although the parasitism of the normal skin was less, the histological changes, i.e. proliferation of macrophages in the dermis and infiltration round hair follicles and sebaceous glands, were quite as well marked as in the Maltese animals. The histological changes in the skin as in other organs are not necessarily related to the intensity of parasitism.

Effect of diet on canine visceral leishmaniasis. The following facts were established as a result of a chance observation on the effect of a fresh meat diet on the disease in dogs.

Dog No. 29. This animal was brought to the laboratory on 26 August 1934 in a state of extreme emaciation. The skin showed uniform seborrhea and there was ulceration on both ears. The dog was not able to bark, could hardly stand, and was not expected to live for more than a few days. Blood smears showed a heavy infection of *Hepatozoon canis*, and microfilariae were found in cover-slip preparations.

The animal was laparotomized and the spleen was found to be very large and soft. Spleen smears showed numerous leishmania, hepatozoa and microfilariae.

28 August 1934. Fourteen *P. major* fed on the animal and eleven became infected. From 2 to 22 September 200 g. of fresh meat was added to the diet. The animal improved and was able to stand. The effect of the fresh-meat diet on the infection rate in sandflies is shown in the following figures:

	No. of sandflies fed	No. positive	%
28. viii.–5. ix.	61	36	57·4
7. ix.–13. ix.	51	16	31·4
13. ix.–18. x.	41	6	14·6

From 28 August to 22 October the fresh meat was removed from the diet and the animal fed on an ample diet of bread and milk in sufficient quantity to keep a normal dog in good health. The animal returned to its former condition, was unable to stand and had frequent attacks of vomiting. The intensity of parasitism in the skin did not return to the former level (before the commencement of the meat diet), for on 18 October thirteen *P. major* fed on the animal and only two became infected. Fresh meat was again added to the diet and the animal again improved and increased in weight, e.g. 14 November, weight 11·8 kg., 3 December, weight 16·5 kg.

During the above observation period pieces of skin were removed at intervals for histological examination. It was found that the proliferation of macrophages in the skin did not diminish during the period in which the animal was kept on a diet of fresh meat, but the numbers of parasites decreased as is shown by the sandfly feeding experiment. It is evident that the macrophages which had previously been unable to digest the leishmania acquired this property under the influence of fresh meat. Further, the absorption of food which had previously been unsatisfactory, as shown by the emaciation on a diet of bread and milk, probably owing to infiltration of the mucosa by macrophages and plasma cells, also changed rapidly under the influence of fresh meat and was associated with an increase in weight.

On 2 December the animal was brought to Jerusalem and kept on a diet of fresh meat and boiled rice. The amount of fresh meat was raised to 500 g./day (from 200 g.) and subsequently up to 1 kg./day. The improvement continued but there was no noticeable change in the external condition of the skin (seborrhea and depilation), and the animal barked very rarely and hoarsely (infiltration of the vocal cords). The number of parasites in the skin increased in spite of clinical improvement, and forty-three out of eighty-seven laboratory-bred *P. major* of Cretan origin fed on this animal between 3 January 1935 and 29 April became infected.

During February 1935 there was an epidemic of enteritis of undetermined origin among the dogs of the animal house in Jerusalem, and two out of five healthy animals died; dog No. 29 was also infected but recovered.

On 3 May the animal became debilitated and showed loss of appetite and diarrhoea. On 5 May the animal died shortly after administration of thymol per os (the immediate cause of death was the entry of thymol into the respiratory passages).

Post-mortem. The animal was well nourished and there was a considerable deposit of fat in the omentum and subcutaneous tissue. There were patches of broncho-pneumonia at the base of the right lung. The spleen was large, hard and fibrous (when first examined by biopsy it was large and soft). Nothing else of note was found on macroscopic examination.

On microscopic examination leishmania were detected in the spleen, but their number was far smaller than on previous examinations. Microfilariae were found in the blood but no adult worms could be discovered though a careful search was made. A scanty *Hepatozoon canis* infection was noted. Histological examination showed the usual skin changes and intense infiltration of the vocal cord with plasma cells and macrophages. Very few parasites were found in the skin in spite of the fact that 50 % of *P. major* infected themselves on this animal during the last two months of its life. As is usual in dogs with relatively few parasites in the skin not only were few macrophages infected but the number of parasites in each infected cell was also small.

Very few parasites were found in the liver or spleen. The liver showed intense proliferation of Kupfer cells, not only within the capillaries but also among the parenchyma cells outside the capillaries. Small masses composed of this type of cells were found scattered throughout the liver. The mucosa of the large and small intestines showed a moderate infiltration with plasma cells and macrophages and very few parasites.

This dog displayed several interesting features. In the first place the feeding of fresh meat to an infected animal apparently *in extremis* brought about a rapid clinical improvement; the withdrawal of fresh meat was followed by a clinical relapse, and a return to the fresh-meat diet was again followed by improvement. The animal's life was prolonged for 8 months by a change in diet without any other treatment. The fresh meat did not remove the infiltrations in the normal skin as was shown by biopsy at various intervals, but the macrophages which had previously been unable to digest the leishmania within them did so under the influence of the new diet and this resulted in a lower infection rate in *P. major*. Finally the skin infection again increased but did not reach its former intensity.

In contrast to the above record that of the following animal is instructive.

Dog No. 71. This animal, which was first seen on 14 July 1934, was well nourished and active but showed seborrhea and almost complete depilation. It was left with the owner till 19 September, when it was brought to the laboratory. It was kept on a diet of bread and milk in quantities sufficient to keep a normal dog in good health. Fresh meat was not given because the effect of this diet on the infection rate in sandflies was already becoming obvious, and it was desired to have as many infected sandflies as possible. The infection rate in *P. major* was as follows:

	No. of sandflies fed	No. positive	%
19. ix.–13. x.	67	31	46·3
16. x.–1. xi.	170	133	80
3. xi.–9. xi.	68	52	76·5

The animal died on 12 January 1935. On histological examination a moderate skin infection was found and a heavy visceral infection. Although parasites were far more numerous in the liver than was the case in dog No. 29, the proliferation of Kupfer cells was far less. Parasites were more numerous in the mucosa and submucosa of the intestines which were more intensely infiltrated than any other organs.

This record can again be contrasted with that of the following animal.

Dog No. 66. It was brought to the laboratory on 12 September 1934 in a state of extreme emaciation. There was extensive ulceration on both buttocks and well-marked seborrhea. The animal was not expected to survive. The external condition of the animal's skin was such that previous experience justified an expectation of an infection rate approaching 100 %. The animal was placed immediately on a diet of fresh meat (200 g./day) and bread and milk.

Between 26 September and 29 October thirty *P. major* fed on dog No. 66 and only five became infected.

There was a marked clinical improvement and the areas of ulceration on the buttocks diminished, but the animal died of exposure on board ship. Parasites in the skin were so scanty that they could not be detected histologically.

The infection rate in *P. major* fed on naturally infected dogs in Canea varied from 2 to 70 %. All the nine infected animals were examined histologically and none (including dogs No. 71 and 29) showed the intense infection of the unbroken skin found in many animals in Malta. In six of the animals the skin infection would have been entirely overlooked if its presence had not been suspected beforehand and a prolonged examination made. It is difficult to make even an approximate quantitative comparison of the degree of skin infection in animals in Malta and Crete, but the difference between the two series was so marked that it appears legitimate to infer that the Cretan strain of *L. infantum* differs slightly from that of Malta. The less intense skin infection produced by the Cretan strain is, however, amply compensated for by the higher infection rate produced by it in *P. major*.

The findings in Canea indicate very clearly the role of house dogs in the maintenance of the human disease. During 1932 a total of forty-five and during 1933 a total of forty-two human cases were reported in Canea. Destruction of street dogs commenced in May 1933, and in 1934 a total of thirty human cases was discovered in the town. The greater part of the cases found in 1934 could only have been caused by sandflies which had infected themselves on house dogs. It is therefore obvious that an attempt to control the disease in human beings by the destruction of dogs cannot succeed unless it includes the detection and destruction of infected house dogs. The diagnosis of the disease in house dogs without external signs of disease can be facilitated by the formol gel test carried out with defibrinated blood instead of serum. This test was positive in all the nine infected animals examined.

THE BEHAVIOUR OF *L. INFANTUM* IN SANDFLIES, PARTICULARLY *P. MAJOR*

The behaviour of *L. infantum* in *P. major* was studied by feeding sandflies of this species on naturally infected dogs, spermophils infected with Greek human strains of *L. infantum*, and Syrian hamsters infected with human strains of *L. infantum* from Malta.

In assessing these experiments it should be emphasized that, as regards digestion, excretion and also fertilization, *P. major* behaves very differently in the laboratory than when under natural conditions. As these differences probably result in corresponding differences in behaviour of flagellates in the insects, laboratory experiments have to be interpreted with caution.

Feeding experiments were carried out on seven naturally infected dogs, and in *P. major* the infection rate varied from 2 % in one animal (one out of fifty-one) to 70 % in another (216 out of 305).

Out of a total of 330 *P. major* infected on dogs and dissected 4–23 days after the infecting feed, twenty-nine had infections confined to the stomach, twelve had infections extending anteriorly into the lower part of the cardia only, and the remaining 289 had infections extending up to the oesophageal valve or further. Six sandflies had infections in the proboscis, and of these three showed infections in the anterior part of the proboscis, i.e. three out of the total number of 330 infected sandflies would inevitably have deposited flagellates into the skin on refeeding and 324 could not possibly have done so. Short forms were only noted on two occasions.

It is to be noted that after 4 days time plays no important part in the distribution of the flagellates in *P. major*. Pure stomach infections were found as late as 10 days after the infecting feed, and proboscis infections were found as early as 5 days and as late as 24 days after the infecting feed. The variations in the distribution of the flagellates in the sandfly depend entirely on the individual sandfly, e.g. of two laboratory-bred sandflies hatched at the same time from the same breeding pot and dissected 10 days after feeding on the same dog, one had an infection confined to the stomach while in the other the flagellates invaded the anterior part of the pharynx.

For reasons previously stated the above findings probably do not represent what would have occurred under natural conditions. The scarcity of short forms of the flagellate, i.e. the type which produces proboscis infections more frequently than others is very noticeable. Both in *P. perniciosus* and *P. sergenti* short-form infections occur in individual females showing no trace of the blood meal which has been completely digested. The latter condition is rarely fulfilled in the case of *P. major* in the laboratory, although it is frequent in this species under natural conditions. It may therefore be inferred on the basis of the above indirect evidence that both short forms and proboscis infections occur more frequently in nature than they have done in the above laboratory experiments.

Sandflies of Cretan origin fed on Syrian hamsters infected with Maltese strains and on a spermophil infected with a Greek strain of *L. infantum* gave the following results:

Hamster no.	No. of *P. major* fed	No. positive	No. of *P. papatasii* fed	No. positive	No. of *P. sergenti* fed	No. positive	No. of *P. chinensis* fed	No. positive
1	124	106	8	0	20	2	3	2
2	46	19	35	0	6	1	—	—
3	8	5	2	2	—	—	—	—
4	8	1	5	0	—	—	—	—
5	7	2	7	0	—	—	2	0
Spermophil	62	42	6	1	11	1	—	—

The behaviour of the flagellates in *P. major* infected on the above animals was similar to that found in the case of sandflies fed on dogs and calls for no special comment. Of a total of 175 infected *P. major* there was a proboscis infection in three, of which two were infected on the spermophil.

NATURAL INFECTION IN *P. MAJOR*

One wild female of *P. major* caught near a house which harboured an infected dog was found naturally infected with flagellates indistinguishable from leishmania. This specimen had no residue of a blood meal in its gut. Its eggs were in their earliest stage of development, but a batch of eggs had been laid before its capture as proved by the presence of granules in the accessory glands (an infallible sign of previous oviposition in sandflies with an empty midgut). The infection was heavy in the midgut and the cardia was choked with flagellates near the oesophageal valve.

Flagellates from this sandfly were rubbed into the broken skin of a spermophil on 7 July 1934. The animal died of pneumonia on 24 November 1934, and was not found infected with leishmania.

TRANSMISSION OF *L. TROPICA* IN CANEA

It has previously been mentioned that though no part of Canea is free from oriental sore the disease is particularly common in the old Turkish town. A sandfly survey showed that in this part *P. papatasii* and *P. sergenti* are common. *P. major* is very rare (five specimens caught during the period under observation), and not a single specimen of *P. chinensis* var. *simici* was found. It appeared therefore that the probable carrier was either *P. sergenti* or *P. papatasii* or both. Feeding experiments were carried out with *P. papatasii*, *P. sergenti* and *P. major*. Laboratory-bred and wild specimens of these sandflies without any trace of a blood meal were used. The insects were fed on non-ulcerating lesions, and cases with the heaviest infections available were used for the feeding experiments. While it is not possible to give a quantitative estimate of the number of leishmania ingested per sandfly, in three of the cases (Nos. 1, 7, and 9) the lesions were so heavily infected that the insects fed on a thick paste of leishmania. The results of the feeding experiments are shown in the following table:

Case no.	No. of *P. sergenti* fed	No. positive	No. of *P. papatasii* fed	No. positive	No. of *P. major* fed	No. positive
1	19	5	19	1	42	8
5	2	0	6	2	1	1
6	2	1	1	0	—	—
7	12	10	9	0	1	0
9	42	36	10	1	11	5

Of the total of fifty-two infected *P. sergenti* all showed an anterior infection from 4 to 11 days after the infecting feed. Short-form infections were found in fifteen (5–11 days after the infecting feed), and of these two had a pure short-form infection. Of the fifteen sandflies which showed the short forms, four (6–9 days after the infecting feed) contained active flagellates in the proximal part of the epipharynx only and three (5–8 days after the infecting feed) contained active flagellates throughout the whole length of the epipharynx up to its distal extremity, and in one of these numerous active flagellates were actually seen escaping from the epipharynx into saline under the microscope. Of the fifty-two infected sandflies three would have inevitably deposited flagellates into any object on which they fed, i.e. in Canea *P. sergenti* readily transmits *L. tropica* by its bite. Actually the infectivity of the local strain of *L. tropica* for *P. sergenti* is not nearly as marked as that of the Baghdad strain of *L. tropica* for the Baghdad strain of *P. sergenti*. Lesions with infections similar to those chosen for the above experiments would be expected to give in Baghdad infection rates approaching 100 % in *P. sergenti* and a high infection rate in *P. papatasii*. It appears that the cases with heavily infected lesions are mainly responsible for the spread of the disease, and other cases with moderate infections or with few parasites (as in old infections) are of very little importance in this respect. The relatively high infection rate produced in *P. major* was unexpected, particularly so since the disease is commonest in districts where this sandfly is almost absent. Of the fourteen infected sandflies of this species twelve had an anterior infection 5 days and more after the infecting feed. There were no short-form infections and no proboscis infections. The difference in infectivity for *P. major* between *L. tropica* and *L. infantum* in Canea is very striking. The latter produced an infection rate of approximately 70 % even when the parasites were so few that they could hardly be detected by histological examination of infected skin, while the former produced a much lower infection rate with lesions containing innumerable leishmania. The very low infection rate produced in *P. papatasii* (much lower than that produced in *P. major*) is very striking and easily differentiates the Canea strain of *L. tropica* from those of Palestine, Tunisia, Algeria, and even Baghdad. It is evident that this sandfly is negligible as a vector of *L. tropica* in Canea.

EXPERIMENTS WITH *L. tropica*

It was previously shown (1932) that there are constant biological differences between strains of *L. tropica* from Baghdad which are transmitted mainly by *P. sergenti* and strains from Palestine which are transmissible both by *P. sergenti* and *P. papatasii*. In one experiment previously recorded (1932) a volunteer spontaneously recovered from a Palestinian strain of *L. tropica* was reinfected with a Baghdad strain, while another who proved refractory to repeated inoculations with Palestinian strains was easily infected with a Baghdad strain.

Attempts were made to infect another volunteer who had been infected with *L. tropica* from wild *P. papatasii* in 1925 and who had recovered spontaneously in 1927.

Experiment No. 1.

7 September 1934. Wild *P. sergenti* No. 110 fed on case No. 6.

14 September 1934. Dissected and found heavily infected. The cardia was choked with flagellates.

Flagellates were inoculated into a scarified point on the left arm of volunteer No. 1.

7 September 1934. Wild *P. sergenti* No. 111 fed on case No. 7.

12 September 1934. Dissected and found heavily infected (long forms).

Flagellates were inoculated into another scarified point on the left arm of the same volunteer.

Result: The inoculations were followed by a typical specific local reaction, i.e. a small scaling papule was produced on each site. The papules were free from parasites. A similar reaction has been repeatedly observed in this case since 1927 after the inoculation of dead flagellates. Apart from the above local reaction the above two experiments have remained negative during an observation period of more than three years.

Experiment No. 2.

19 September 1934. *P. sergenti* No. 143 (laboratory bred) fed on case No. 9.

25 September 1934. Refed on volunteer No. 2.

29 September 1934. Dissected and found heavily infected (long forms).

Result: Negative during an observation period of three years.

Experiment No. 3.

24 September 1934. *P. sergenti* No. 160 (laboratory bred) fed on case No. 9.

1 October 1934. Dissected and found heavily infected. The whole length of the epipharynx was full with very active short forms. Flagellates from the proboscis were introduced into a scarified point on the left arm of

volunteer No. 2. Flagellates from the stomach were introduced into another point. Flagellates from the cardia were introduced into a scarified point on the left thigh of another volunteer (No. 3).

Result: The above three inoculations have remained negative during an observation period of three years.

Experiment No. 4.

7 October 1934. *P. sergenti* No. 192 fed on case No. 9.

13 October 1934. Dissected and found heavily infected in the cardia (short forms). Proboscis negative. Flagellates inoculated into a scarified point of left arm of volunteer No. 2.

Result: At the beginning of April 1935 a small papule was noted at the site of the inoculation. No parasites were found in smears.

By October 1935 the papule had increased in size and formed an infiltrating mass about ½ cm. in diameter. Numerous leishmania were found in smears. Cultures were made and the strain has been maintained and observed in the laboratory up to the present. After about 6 months spontaneous recovery took place.

The behaviour of this strain was further studied by feeding *P. papatasii* of Palestinian origin on emulsions of culture through a membrane. In its behaviour in *P. papatasii* this strain contrasted very strikingly with a Palestinian strain originating from a naturally infected *P. papatasii*. This is well illustrated by the following figures.

Of fifty-one laboratory-bred *P. papatasii* from Palestine fed on an emulsion of the Canea strain of *L. tropica* in 50 % inactivated rabbit serum (1600 flagellates per 0·1 c.mm.) six were subsequently found infected.

Of thirteen laboratory-bred *P. papatasii* from Palestine fed on an emulsion of Palestinian *L. tropica* in 50 % inactivated rabbit serum (1200 flagellates per 0·1 c.mm.) all the thirteen were found infected.

With regard to infectivity for *P. papatasii* the difference between the Canea, Palestinian, Algerian, and Tunisian strains of *L. tropica* is as great as that previously shown to exist between Palestinian *L. tropica* and *L. donovani* from India and *L. infantum* from the Mediterranean basin.

THE DISTRIBUTION OF SANDFLIES AND VISCERAL LEISHMANIASIS

In order to emphasize the gaps in the data available and to indicate lines for further research it will be necessary to review our knowledge of the distribution of sandflies and leishmaniasis throughout the world.

In the Mediterranean basin *P. perniciosus* is the main carrier in North

Africa, France and Italy. It has been recorded from Spain, but its actual distribution in the peninsula is not known. Future research must decide whether this species only or *P. ariasi* or both transmit visceral leishmaniasis in Spain and Portugal.

The eastern limits of the distribution of *P. perniciosus* appear to be rather sharply defined. While it is common in Rome, Naples, southern Italy and Sicily it has not yet been recorded from north-eastern Italy. During a brief survey carried out in Terramo in the Abruzzi *P. perfiliewi* and *P. papatasii* were found to be very common, and not a single specimen of *P. perniciosus* was discovered.

P. perniciosus var. *tobbi* has a wide range of distribution extending from the Adriatic to the shores of the Caspian and in the south to Palestine, but this variety is certainly not the main carrier throughout its zone of distribution, for within this region there are pockets where it is absent though visceral leishmaniasis is common, e.g. Canea.

In the Near East *P. major* and *P. major* var. *syriacus* are the main carriers of visceral leishmaniasis. Other species of the major group play a minor role, although they can all be infected by feeding on infected Syrian hamsters. Actually there are small centres where *P. major* is the only possible carrier, other species of the major group being absent, e.g. Beit Iksa, a small endemic centre near Jerusalem.

In Senegal, where a few cases of canine visceral leishmaniasis have been recorded, the carrier cannot as yet be indicated, for very little is known of the sandflies which feed on mammals in tropical Africa. Only two species of the major group have so far been recorded in Africa south of Egypt, i.e. *P. langeroni* in the Sudan and Abyssinia and *P. katangensis* in the Congo, but nothing is known of their bionomics and no experimental work has been carried out. *P. langeroni* should for the present be regarded as the probable vector in the Sudan.

In India the position is quite clear, *P. argentipes* being the vector of *L. donovani* in that area.

In China *P. chinensis* has been considered to be the vector of visceral leishmaniasis, and recently Jung Sun and others (1936) have found this sandfly naturally infected in a Chinese endemic centre, but it cannot be stated with certainty that this species is the only vector. Patton and Hindle (1927) have pointed out that in China *P. chinensis* is active from the end of May to the beginning of July. Hindle (1928), on the other hand, has recorded the case of a child which was born in November and found infected in the following March before the commencement of the sandfly season. Hertig in a personal communication noted that *P. chinensis* is very common in parts of Peking where the disease is rare and drew attention to the limited seasonal distribution of this species. For these and other reasons, particularly the finding of leishmania in smears from tonsils of infected human beings, Forkner and Zia came to the conclusion that in China transmission is not by sandflies but by contamination. As the theory of transmission by contamination has been discussed in a previous communication (1935) and shown to be untenable, it need not be considered any further here. More exhaustive entomological research will probably show that in China, as elsewhere, the epidemiology of visceral leishmaniasis can be completely explained on the basis of transmission by sandflies, either by *P. chinensis* itself or by some other hitherto unrecorded local species of the *major* group.

In Turkestan the epidemiology of visceral leishmaniasis is very similar to that of the Mediterranean basin. Dogs are frequently infected and serve as a reservoir for the infection of other dogs and man. This type of the disease extends irregularly from Central Asia to the Caucasus, Asia Minor and throughout the whole Mediterranean region to Portugal. The following species of the *major* group have been recorded from Turkestan: *P. major*, *P. chinensis*, *P. wenyoni*, and *P. kandelaki*. While there is as yet no local experimental evidence incriminating one of the above species more than another, the similarity of the disease in Turkestan to that in the Mediterranean region indicates a common carrier, i.e. *P. major*.

No information is available regarding the transmission of the recently discovered visceral leishmaniasis of the New World. No sandflies of the *major* group have as yet been found in Brazil, where autochthonous cases of the disease have been reported. The known sandflies of the New World are very different from those of the Old World with the exception of two Brazilian species, *P. brumpti* and *P. troglodytes*. Neither of these species has affinities with the *major* group, but both, particularly *P. brumpti*, are related to *P. papatasii*, which is more closely related to them than to any known Old World species. The fact that *P. papatasii* can be infected with *L. infantum* and *L. donovani* if fed on very heavily infected animals suggests the advisability of carrying out experiments with *P. brumpti* and *P. troglodytes*.

SUMMARY

The epidemiology of leishmaniasis in Canea is discussed. Human visceral leishmaniasis, canine visceral leishmaniasis, and the sandfly *Phlebotomus major* have an identical distribution in Canea. This is the only sandfly in

Canea of importance from the point of view of the transmission of visceral leishmaniasis. *P. perniciosus* var. *tobbi* and *P. perfiliewi* are absent, and *P. chinensis* var. *simici* is not common.

The bionomics of *P. major* in Canea are discussed.

The male of *P. laroussei* is described and the relation of this species to *P. vesuvianus* and *P. canaaniticus* is discussed. *P. vesuvianus* is considered to be a synonym and *P. canaaniticus* a variety of *P. laroussei*.

The clinical condition of naturally infected dogs is improved and the infection rate in *P. major* fed on these animals reduced by placing them on a diet of fresh meat without any further treatment.

It has been noted that naturally infected dogs have difficulty in barking owing to infiltration of the vocal cords with macrophages and plasma cells.

P. major, *P. chinensis* var. *simici*, *P. sergenti*, and *P. papatasii* were infected with *Leishmania infantum* by feeding on naturally infected dogs, on Syrian hamsters and a spermophil. The infection rates in *P. sergenti* and *P. papatasii* very were low as compared with that in *P. major*.

P. sergenti, *P. papatasii* and *P. major* were infected with *L. tropica* by feeding on cutaneous lesions in human beings in Canea. The infection rate in *P. papatasii* is very low as compared with that in *P. sergenti*. *P. papatasii* in Canea plays no significant role in the transmission of *L. tropica* of which *P. sergenti* appears to be the main vector.

The Canea strain of *L. tropica* differs biologically from that of Palestine. In contrast to the latter it produces a very low infection rate in *P. papatasii* both in Greece and Palestine.

A human being was infected with *L. tropica* by the cutaneous inoculation of flagellates from an artificially infected *P. sergenti*.

The general distribution of sandflies of the *major* group with relation to visceral leishmaniasis in the Old World is discussed.

ACKNOWLEDGEMENTS

We have to thank Dr Norman White, Professor Joakimoglou and Dr Papantonakis for their interest and help, without which this work would have been impossible.

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576.354.4 : 595.725 *Callimantis*

A new and anomalous type of meiosis in a mantid, *Callimantis antillarum* Saussure

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[Plates 30–32]

1. Introduction

The general course of meiosis in the Orthoptera is remarkably constant from species to species, and even from one suborder to another. This is at any rate true of meiosis in the male sex; owing to technical difficulties, oogenesis has been studied in only a few species, but there seems no reason to believe that it is less uniform than spermatogenesis.

Such deviations from normality as do occur seem to be of two main kinds. In some species the middle part of the prophase of the first meiotic division (pachytene, and sometimes diplotene as well) are replaced by a "diffuse stage" during which the chromosomes become almost impossible to fix and stain. Such a stage is apparently present in the peculiar orthopteran *Schizodactylus monstrosus* (McClung and Asana 1933, 1935). In other species the chiasmata which usually occur more or less at random along the length of the meiotic chromosomes are localized in certain definite regions. Localization of chiasmata is seen in its most extreme form in species of the genus *Mecostethus* (Acrididae), where only one chiasma is usually formed in each bivalent, just near the spindle attachment (White 1936).

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Phenomena such as the occurrence of supernumerary chromosomes (Carroll 1920), inversions (Darlington 1936), or unequal bivalents (Carothers 1931) cannot be regarded as alterations in the meiotic mechanism as such; they are merely peculiarities of individual chromosomes.

The present paper deals with the spermatogenesis of a mantid in which the normal meiotic mechanism is entirely different from that found in all other Orthoptera hitherto investigated. No chiasmata are formed at any stage, so that the first meiotic division is reductional for all parts of all the chromosomes. In this respect *Callimantis* resembles the male *Drosophila* in which, according to Darlington (1934), no chiasmata are formed in the autosomes and in which no genetical crossing-over normally occurs. The similarity between the cytological phenomena involved appears, however, to be a rather superficial one and the underlying mechanism quite different, although the final result is the same.

The spermatogenesis of five species of mantids (three species of *Paratenodera*, *Mantis religiosa* L. and *Stagmomantis carolina* Johansson) has been studied by previous workers (Oguma 1921; King 1932). These authors did not find any peculiarities of meiosis comparable to the conditions in the present species. On the other hand, they did find an unusual sex-determining mechanism of the $X_1X_1X_2X_2$ (female) and X_1X_2Y (male) type. The present species is simply XO in the male. It thus differs both in its meiotic mechanism and in its sex chromosomes from the Mantids previously investigated.

2. MATERIAL AND METHODS

Callimantis antillarum is a small mantid belonging to the same subfamily (the Mantinae) as the five species previously studied. In all probability it is the only species of the genus, which is a rather isolated one. The three male individuals on which my work is based were collected at Port-au-Prince (Haiti) by Dr K. W. Cooper, who handed the material over to me for investigation. I cannot thank him too warmly for his generosity in this respect. The material had been fixed for 4 hr. in Allen's Bouin. It was subsequently sectioned at 15μ and stained by Newton's gentian violet method. The species was identified for me by Mr J. A. G. Rehn of Philadelphia, whom I wish to thank for his kindness.

3. OBSERVATIONS

The chromosomes were studied at mitosis in the spermatogonial divisions and also in the somatic divisions of the interstitial cells of the testis. The latter are polyploid in many Orthoptera, but in the present species there can be no doubt that they are diploid. Typical metaphase plates are shown in figs. 1 and 2 (Plate 30). There are seventeen chromosomes, all of which are V-shaped. It will be shown subsequently that one of the smaller V's is the *X*-chromosome. Neither in the spermatogonial nor in the somatic metaphases is it distinguished by any heteropycnosis, so that it cannot be identified with certainty at this stage. There are some size differences between the chromosomes, but in most cases they are not sufficiently great to enable one to pick out homologous chromosomes with any certainty. In a number of chromosomes the two arms are almost equal in length, but in one of the large pairs and also in one of the smaller ones, one of the arms is a good deal shorter than the other.

At the spermatogonial telophases the *X*-chromosome shows a certain amount of positive heteropycnosis, and in the interval between the telophase of one division and the prophase of the next it shows the same phenomenon rather clearly. The general appearance is not unlike the heteropycnosis of the *X* at the same stage in the long-horned grasshoppers (White 1938), but no clear relic spirals have been seen here. At one stage (which I interpret as a very early prophase) the heteropycnotic *X* takes up a position in the centre of the nucleus, surrounded by all the autosomes, which lie on the inside of the nuclear membrane.

In the premeiotic stages the *X* is still heteropycnotic (fig. 3, Plate 30). It is doubled up on itself and appears to be outside the main nucleus, possibly enclosed in a separate vesicle. It was unfortunately not possible to study the leptotene stage in detail, but zygotene pairing appears to take place quite normally. A zygotene nucleus is shown in fig. 4, Plate 30, but it must be pointed out that the detailed structure of the chromosome threads is a great deal clearer than appears in the photograph; in well-fixed nuclei one can see that the threads are paired in some parts and not in others. The *X*-chromosome is now much closer to the autosomes; it is a pear-shaped body lying on the periphery of the main nucleus (whether inside or outside the nuclear membrane cannot be determined). By following through the stages from preleptotene to zygotene it was possible to determine that the two ends of the *X*-chromosome lie together at the narrow end of the "pear", the broad end being the point of flexion.

The early pachytene nuclei are quite normal, and do not differ in appearance from the corresponding stages in a long-horned grasshopper such as *Tettigonia* (Mohr 1916). Such an early pachytene nucleus is shown in fig. 5 (Plate 30); it will be seen that the pairing of the homologous chromosomes has been completed. The *X*-chromosome is still heteropycnotic, and lies on the periphery of the nucleus. In late pachytene (figs. 6 and 7, Plates 30 and 31) the middle regions of the chromosomes show slight heteropycnosis; that is to say that the chromomeres (blocks of chromatin) on either side of the spindle attachment are more bulky than those towards the ends of the chromosomes. The appearance is rather similar to that figured by Darlington (1934) for the plant *Agapanthus*. Such a slight heteropycnosis of the region round the spindle attachment is by no means rare in the pachytene chromosomes of the Orthoptera—it is also seen very clearly in the diplotene bivalents of *Mecostethus* spp.

At each end of a late pachytene bivalent is a single rather distinct chromomere which stains darkly. These terminal chromomeres appear to be present in all the autosomes. All the terminal chromomeres in the nucleus show a tendency to become approximated to one another in two or more groups. The result of this is that the chromosomes become polarized as in *Chorthippus* (Darlington 1936; figs. 8–11, Plate 2). If the two ends of each bivalent are attracted to opposite polarization centres the eight bivalents become stretched across the nucleus rather like the staves of a barrel; on the other hand some of the chromosomes usually form loops with both ends approximated to the same polarization centre. Thus there may be two "bouquets" at opposite sides of the nucleus. In one cell three autosomes formed loops at one side of the nucleus, and the *X* was also attracted to the same point; four autosomes formed loops at the other side of the nucleus and the eighth bivalent was stretched between the two polarization centres. A late pachytene nucleus in which most of the bivalents were stretched across from one side to the other is shown in fig. 6; unfortunately only one chromosome is in focus from end to end.

After pachytene the bivalents pass directly into prometaphase (fig. 8). There are no stages which correspond in any way to diplotene or diakinesis. In some instances all the stages from pachytene to metaphase are present in the same testis-lobule, so that one can be sure that no stages have been missed. The prometaphase chromosomes only differ from the late pachytene ones in being thicker and in the fact that the chromomeres are no longer distinct. Each prometaphase bivalent consists of two homologous chromosomes paired throughout their whole length, and in some cases slightly twisted round one another (these twists can also be seen at late pachytene). There is no evidence that the chromosomes have undergone any split at this stage; if they have it is an invisible one, since the only "split" which can be seen is the one between the two chromosomes. The X has lost its heteropycnosis by this time, but it can be distinguished from the autosomes, because it is univalent and not composed of two paired chromosomes.

In the first metaphase (figs. 9–13, Plates 31, 32) the structure of the bivalents can be seen very clearly. In polar view (figs. 9–11) they are V-shaped, with the spindle attachment at the junction of the two arms. Their general appearance is very similar to the bivalents of *Mecostethus* at the same stage. In at least one of the bivalents, however, (the one in the lower left-hand corner of fig. 9 and in the lower right-hand corner of fig. 10) the arms are of unequal length.

It is very difficult to be certain whether the chromosomes are split in metaphase, but it seems probable that they are. In most cases the outline of the chromosome stains darkly, while the centre remains relatively clear. This appearance probably indicates the existence of a split, since the dark outline does not extend round the ends of the chromosomes as one would expect it to do if it were merely an artefact or optical effect (these details are best seen in the lower bivalents of fig. 11).

As one focuses up and down on a bivalent in polar view the region containing the spindle attachment is the last to disappear; the reason for this is seen when one examines side views of bivalents. In these side views (figs. 12 and 13) it is clear that the spindle attachments have already begun to separate. Perhaps it would be better to call this stage "early anaphase", but since no metaphases were found in which the spindle attachments had not begun to separate it is probable that they begin to move apart at the very beginning of metaphase, or even earlier, at the end of prometaphase.

The characteristic "hollow cross" shape of the metaphase bivalents might be thought to indicate the presence of a chiasma if the previous history of the chromosomes were not known. Actually the appearance is entirely misleading, as in the case of the autosomes in the male *Drosophila* at the same stage (Darlington 1934, fig. 35).* The cross cannot indicate a chiasma, since (1) the chromosomes have a median spindle attachment at mitosis, (2) the two horizontal arms of the cross are unequal in length in some bivalents, and hence not homologous.

The small V-shaped X-chromosome usually attaches itself to the spindle at some point between the equatorial plane and one of the poles of the spindle. It is consequently difficult to find nuclei in which it can be photographed in the same focal plane as the autosomes. It is shown in fig. 10 (somewhat out of focus) and again in fig. 13, Plate 32.

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* The two bivalents in this figure of Darlington's have been misplaced, so that they do not correspond to the legend beneath. The one on the left should be on the right and vice versa.

The first anaphase is an extremely simple and characteristic stage (figs. 14, 15, Plate 32). The *X* goes undivided to one or other of the poles. The spindle attachments of the autosomes separate farther and farther apart, dragging the chromosomes after them. At this stage the split down the length of the chromosomes becomes visible; it is not easy to photograph, but is seen in the left-hand bivalent of fig. 14. This figure shows three bivalents in which the arms are subequal in length. In the two bivalents in which the arms are markedly unequal the ends of the longer arms remain in contact for some time after the ends of the shorter arms have parted company.

There appears to be nothing unusual about the interkinetic nuclei. The chromatids are separated, and only held together at the spindle attachment, as is the rule in most organisms at this stage. The *X* usually shows some degree of positive heteropycnosis in those interkinetic nuclei in which it is present (fig. 16, Plate 32). The second meiotic metaphases are naturally of two kinds, according to whether they contain an *X* or not; the plus-*X* metaphases have nine chromosomes, the minus-*X* ones only eight (figs. 11, 12). The chromosomes at this stage are small V's as in other mantids (King 1932). There is nothing unusual about the anaphase of the second meiotic division. Two diploid second metaphases (out of a total of several hundred) were observed—they presumably resulted from failure of the two groups of chromosomes to separate at the anaphase of the first meiotic division.

4. Discussion

The meiosis of *Callimantis* is so unlike the other types of abnormal meiosis found in insects that it seems useless to attempt a general comparison. In the complete absence of chiasmata it resembles the meiosis of the male *Drosophila*. According to Darlington's general theory of pairing at least one chiasma must be formed in every bivalent if the two chromosomes of which the bivalent is composed are not to fall apart after pachytene. In order to explain the case of the male *Drosophila* Darlington (1934) suggested that a "residual attraction" of the same nature as the somatic-pairing force is responsible for the continued association of the homologous chromosomes after pachytene. The explanation is simple and logical in the case of *Drosophila*, where somatic pairing is seen at mitosis; there is no reason to believe, however, that it applies in the present instance, since there is no sign of somatic pairing in *Callimantis* either in the spermatogonial metaphases or in those of the interstitial cells. It seems probable, therefore, that the chromosomes remain associated as bivalents right up to metaphase simply because the split (which normally appears at the end of pachytene) is, in *Callimantis*, delayed until later. Possibly when the split does take place (at metaphase or shortly before) the whole bivalent is enclosed in a "pellicle" which holds the chromosomes together until they are pulled apart at anaphase. The fundamental difference between the meiosis of *Callimantis* and that of other organisms is undoubtedly the complete absence of the usual stages from the latter part of pachytene to prometaphase and the complete absence of chiasmata is a consequence of this. It is very unfortunate that no material of the female is available for study, but since in other insects which have an abnormal meiosis in the male the corresponding process in the female follows the usual course, there is no reason to expect anything out of the ordinary in the female *Callimantis*.

The relation between the sex-determining mechanism of *Callimantis* and that of the other Mantinae must remain obscure until other species of the group have been studied. Possibly the XO condition in *Callimantis* is primitive in the sense that the other mechanism has been derived from it—or both may have been derived from an XY mechanism. In its general shape and in its marked heteropycnosis the "X" of *Callimantis* resembles the "Y" of the other species; on the other hand it shows no resemblance to either the "X_1" or the "X_2" of *Paratenodera, Mantis* and *Stagmomantis*.

SUMMARY

The meiosis of the male *Callimantis* takes place in a manner which is entirely unique. No chiasmata are formed and the first meiotic division is consequently reductional for all parts of the chromosomes. The usual diplotene and diakinesis stages are completely absent. Unlike other mantids hitherto studied this species is XO in the male.

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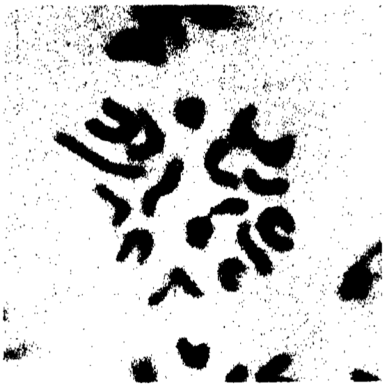

Fig. 1

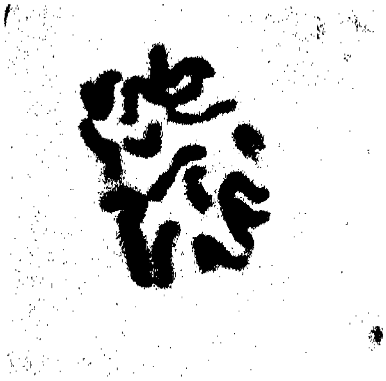

Fig. 2

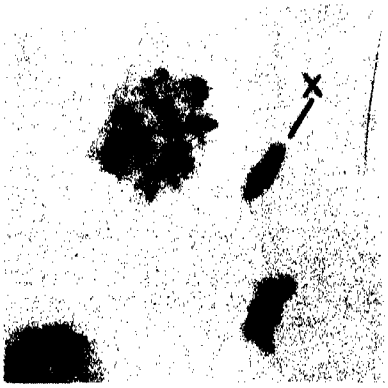

Fig. 3

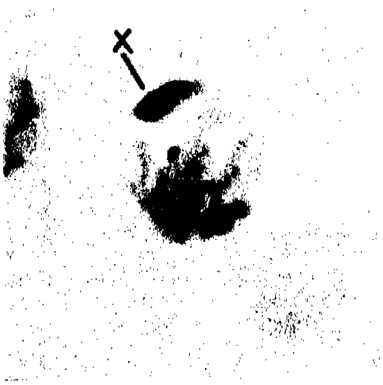

Fig. 4

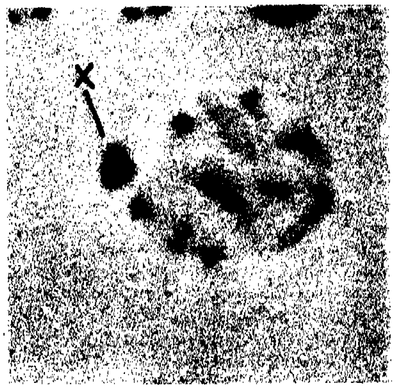

Fig. 5

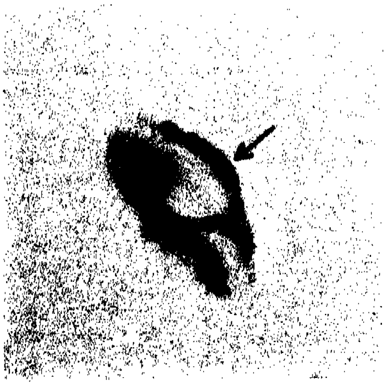

Fig. 6

(*Facing p.* 522)

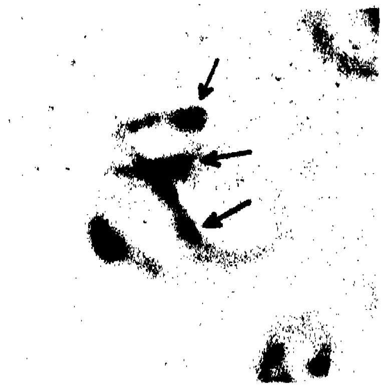

Fig. 7

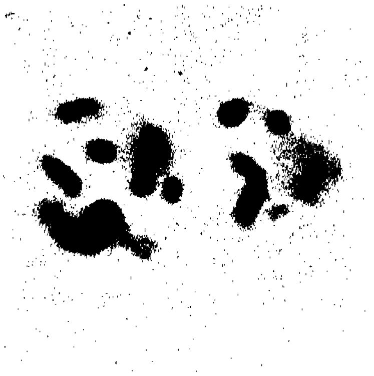

Fig. 8

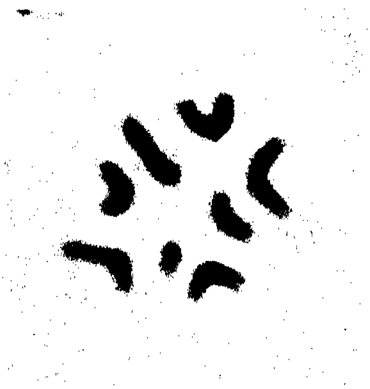

Fig. 9

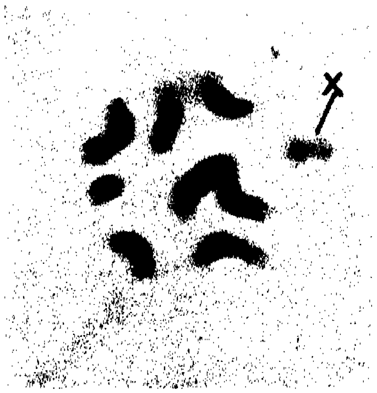

Fig. 10

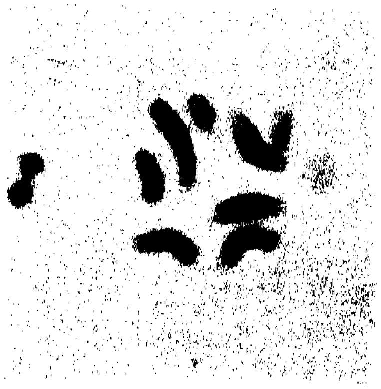

Fig. 11

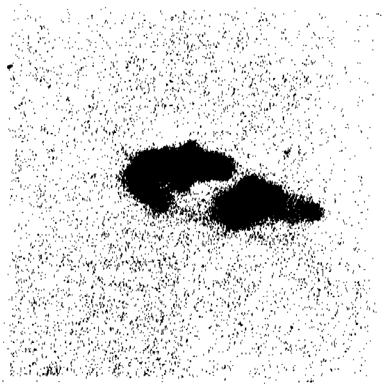

Fig. 12

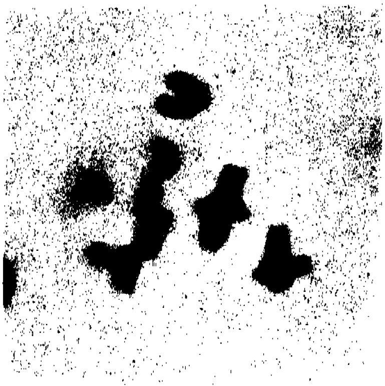

FIG. 13

FIG. 14

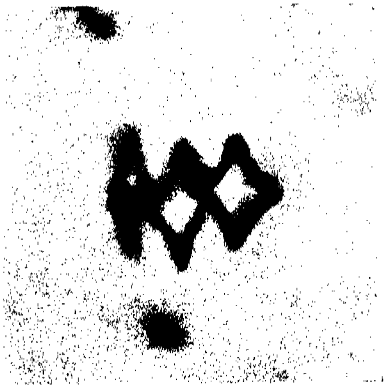

FIG. 15

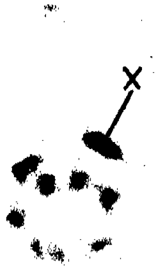

FIG. 16

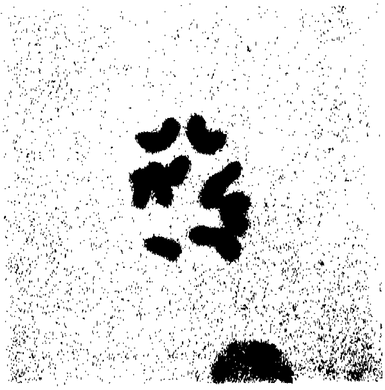

FIG. 17

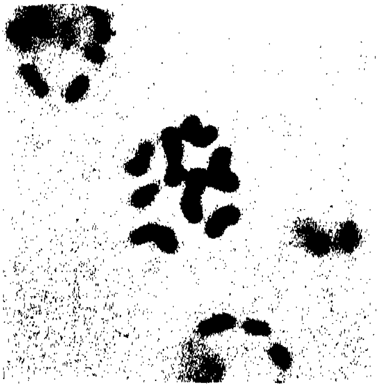

FIG. 18

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DESCRIPTION OF THE PLATES

(All photomicrographs magnified × 2760)

Plate 30

Fig. 1. Spermatogonial metaphase. All 17 chromosomes visible.

Fig. 2. Somatic metaphase in one of the interstitial cells of the testis. Only 16 chromosomes visible (the 17th is out of focus).

Fig. 3. Preleptotene cell showing the heteropycnotic *X*-chromosome lying in a separate vesicle.

Fig. 4. Zygotene nucleus.

Fig. 5. Pachytene nucleus. The *X*-chromosome is still heteropycnotic, but its vesicle appears to have fused with the main nucleus.

Fig. 6. Late pachytene nucleus in the polarized stage. The bivalent on the right is stretched across the nucleus. The arrow indicates the heterochromatic part around the spindle attachment.

Plate 31

Fig. 7. The same stage, showing the *X* and the heteropycnotic regions of three autosomal bivalents (indicated by arrows).

Fig. 8. Two cells in prometaphase of the first meiotic division.

Fig. 9. Metaphase of the first meiotic division (polar view). All the eight bivalents can be seen, but the *X* is out of focus.

Fig. 10. The same. All eight bivalents visible. The *X*-chromosome is the faint bipartite body on the right-hand side.

Fig. 11. The same. *X* not visible.

Fig. 12. Metaphase of the first meiotic division (side view). To show the detailed structure of a bivalent at this stage.

Plate 32

Fig. 13. The same, showing the structure of two bivalents. The *X* can also be seen at the top of the figure.

Fig. 14. Anaphase of the first meiotic division (side view). Three bivalents visible.

Fig. 15. A slightly later anaphase stage.

Fig. 16. Interkinesis. The *X* is heteropycnotic.

Fig. 17. Metaphase of the second meiotic division (plus-*X* class).

Fig. 18. The same (minus-*X* class).

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The function of the ampullae of Lorenzini, with some observations on the effect of temperature on sensory rhythms

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[Plates 33–36]

Introduction

The recognition of the morphological and developmental relationship of the vertebrate auditory organ and the lateral-line system of fishes and aquatic Amphibia rests on the foundation of a large volume of comparative researches. The main outlines of this generalization were already laid down forty years ago, and Cole's work on the cranial nerves and lateral sense organs of fishes (1898) contains a comprehensive treatment of the history of the subject. The acustico-lateral or neuromast system embraces, in addition to the labyrinth and the lateral-line canals, the pit organs found to a greater or less extent in most fishes, the vesicles of *Torpedo,* and the ampullary canal system of Selachians and Holocephali. Concerning these Cole wrote: "The history of our knowledge of the phylogeny of the sensory canals is coincident with three discoveries—the discovery that the 'mucus' canals contain sense organs, the discovery of Savi's vesicles, and the discovery of the ampullae of Lorenzini.... We now know that all three types belong to the lateral line system, and I shall suggest that they represent three stages in the development of a canal—the most superficial condition, represented by the pit organs and Savi's vesicles; the full development, represented by the canal; and the intermediate type, forming neither a Savi vesicle nor yet a canal, represented by the ampullae of Lorenzini" (p. 187).

This conception has remained valid to the present day. The ampullae of Lorenzini, with which I am here principally concerned, are briefly described in current text-books as transitional or specialized neuromasts, and the implication always is that structurally and functionally they do not differ significantly from the neuromasts of the lateral-line canals. For example, in their recent exhaustive treatise on the vertebrate nervous system Kappers, Huber and Crosby (1936) state with reference to the lateral-line canals, the Savi vesicles and the ampullae of Lorenzini: "thus in the various animals there is a transition between an open and a closed system for perceiving vibrations" (p. 438).

Histological structure. A careful study of the literature leaves one with the impression that the unqualified inclusion of the ampullae of Lorenzini in the lateral-line system is unwarranted. In contrast with the very extensive and unanimous evidence on the question of the identity of lateral-line canal neuromasts and the organs of the labyrinth, the relatively few investigations devoted to the structure and innervation of the ampullae of Lorenzini have yielded controversial, and even contradictory results. In the ampulla there are two types of cells, and these were described by Merkel (1880) as pear-shaped sense cells with sense hairs, and supporting cells. It was Merkel's description which established the Lorenzini ampullae as members of the neuromast system. Peabody (1897) could find no sense hairs, and questioned the sensory function of the ampullae. Retzius (1898) agreed with Peabody concerning the absence of sense hairs, but regarded the pear-shaped cells as secondary sense cells, without, however, committing himself as to their probable function. Brandes (1898) advanced a totally different interpretation, describing the pear-shaped cells as gland cells concerned with the secretion of the jelly which fills the ampullary canals, while the pyramidal supporting cells of Merkel were, in his view, sensory. Metcalf (1915, 1916) found no sense hairs, but, like Retzius, ascribed a sensory function to the large pear-shaped cells. In the most recent contribution to the subject Dotterweich (1932) revives Brandes' interpretation. A thorough and extensive histological study leads him to affirm with conviction that the pear-shaped cells secrete the jelly, while the pyramidal cells are the terminal structures of sensory fibres, and are therefore primary sense cells. Dotterweich's work, in my opinion, carries great conviction, but, in view of so much disagreement, his conclusions must await independent confirmation before they can win final acceptance. If Dotterweich is right, the ampullae of Lorenzini are not neuromast organs at all, and our views concerning their morphological relationships will have to be profoundly modified.

Innervation. It is generally stated that the ampullae of Lorenzini are innervated by lateralis fibres. A simple dissection suffices to show that their innervation is closely associated with that of the lateral-line canals. The superficial ophthalmic, buccal and hyomandibular branches of the facial nerve carry the nerve supply to all the groups of ampullae as well as to all the lateral-line canals of the head. Norris (1929) says: "there was in no instance any doubt as to the exact innervation of an ampulla. Each ampulla is innervated by one or more latero-sensory fibres that enter the brain in the roots of the facial nerve, never the trigeminal nerve. They terminate in the lobus lineae lateralis and possibly in the acusticum also." Nevertheless Allis (1901), whose work on the lateralis system is of classical importance, and who is, so far as I know, the only author who has studied the development of the ampullae, gave it as his opinion that they are homologous, not with neuromasts, but with that other group of surface sense organs whose development in *Amia* he had previously described (1889): the terminal buds of ganoids and teleosts. It was Merkel (1880) who had first drawn a sharp distinction between these "Endknospen" and the organs of the lateral-line system, and Herrick (1901) showed that in structure and innervation they are similar to the taste-buds of the mouth, and recognized them as constituting an entirely distinct sensory system, the taste-bud system, whose nerves form a part of the visceral sensory component, and terminate centrally in the fasciculus communis (1903). These organs Herrick (1902) showed experimentally to be gustatory receptors.

Herrick (1903) rejected Allis's contention that the ampullae of Lorenzini of elasmobranchs are related to these superficial taste-buds of ganoids and teleosts. His objection was based, not on any positive evidence concerning the innervation of the ampullae, but on his belief in their histological identity with neuromast organs. His point of view is revealed in the wording of his conclusion: "We can however advance with confidence at least this negative conclusion, that the terminal buds have not been derived from *ampullary organs or any other members of the acustico-lateralis system*" (italics inserted). If Dotterweich's histological findings had been available to Herrick at the time, it is probable that his reaction to Allis's ideas would have been more favourable.

It is not my purpose to advance any definite view as to the relationships of the ampullae of Lorenzini, for I have not studied them histologically or neurologically. But from the functional aspect, as will presently appear, they belong to an entirely different sense modality from the acustico-lateral system, and this fact, taken in conjunction with the uncertainty that characterizes the problems of their histological structure and innervation, calls for a profound revision of our conceptions concerning their morphological and physiological meaning.

Disposition and function. Quite apart from questions of histological structure and innervation the appearance of the ampullary canals of Lorenzini is strikingly different from that of the lateral-line canals. The smooth thin-walled ampullary tubes, filled with a remarkably viscous jelly, lie free under the skin, and lengths of several centimetres can be easily withdrawn. The lateral-line canals lie *in* the skin, and cannot be freed from the connective tissue in which they are embedded. The ampullary canals radiate in all directions from five bilateral groups of ampullae, whose disposition in *Laemargus* has been figured by Ewart (1892), and this figure has found its way into numerous text-books and is reproduced also by Dotterweich (1932). In *Raja batis* Ewart and Mitchell (1892) described a similar arrangement, and I have found that *Raja clavata*, *maculata*, *microcellata* and *brachyura*, as well as *Scyllium canicula* display essentially the same disposition. The ampullae lie together in large clusters around the arborizations of the nerve. They have been picturesquely described as "bunches of grapes". The whole group is contained in a capsule of fibrous connective tissue which is perforated by the ampullary tubes, and inside the capsule the ampullae are held in a delicate, almost fluid, stroma. Fig. 1 (Plate 33) is a photograph of a few of the hyomandibular ampullae of *Raja*, and shows their irregular contour, their connexion with the branches of the nerve, and the smooth tubes of jelly leading from them. The scale is 5 mm. The tubes lie side by side over the surface of the muscle and terminate peripherally at small pores in the skin through which the jelly can be easily squeezed out. Most of the surface area of the head is covered with ampullary tubes.

When one contemplates the remarkable elaboration of the lateral-line canal system and of the ampullary canal system in a ray it is difficult, indeed I would say impossible, to believe that they do not perform entirely separate and distinct sensory functions. Those who have speculated as to the function of the ampullae of Lorenzini have for the most part been preoccupied with their histology and innervation, questions concerning which, as I have shown, no final statement is as yet possible. They have been guided by their belief in the homology of the ampullae with neuromast organs, and have assigned to them a vaguely mechanical function: a receptivity to vibrations, pressure and the like. Very few authors have attempted an experimental approach to the problem. Parker (1909) and Metcalf (1915) have observed muscular movements in response to mechanical stimulation of the ampullae, and Dotterweich (1932) injected distilled water subcutaneously into dogfish and inferred from the restless behaviour of the injected animals that the jelly in the ampullary canals had swelled and had stimulated pressure receptors in the ampullae. It may safely be said that no satisfactory method has yet been applied to the investigation of the sensory function of the ampullae of Lorenzini.

EXPERIMENTAL INVESTIGATION

My recent physiological study of the lateral-line canals (Sand 1937) disclosed the fundamental identity of the underlying mechanism of the lateral-line receptors and of the receptors of the semicircular canals of the labyrinth. It demonstrated also the extreme vibrational sensitivity of the lateral-line system. With the experience gained in that work it was a relatively simple matter to test the ampullae of Lorenzini, and to see whether their activity bore any relation to that of lateral-line receptors. To accomplish this it was necessary to separate the nerve fibres supplying the ampullae from the accompanying lateral-line fibres. The anatomical relations are such that although it is easy to find long slender nerves composed entirely of lateral-line fibres, every nerve branch which innervates a group of ampullae carries also one or more bundles of fibres which pass round or between the ampullae and continue their course to terminate in a lateral-line canal. This is so, at least, in rays; *Scyllium* was less thoroughly examined. The mandibular ampullae are the most convenient to work with. They are a small compact group lying under the skin of the lower jaw just below the corner of the mouth. Their tubes extend in brush-like formation mesially, and terminate in pores scattered over the skin below the lower lip. Their nerve is an offshoot of the great hyomandibular branch of the facial which carries the nerve supply to the hyomandibular lateral-line canal, the large hyomandibular group of ampullae, as well as the motor innervation to some of the jaw muscles. This branch leaves the hyomandibular nerve a short distance above the hyomandibular ampullae, curves downward between the first branchial chamber and the jaw muscles, and, just before it enters the capsule of the mandibular ampullae, gives off a branch which runs along the edge of the capsule to innervate the mandibular lateral-line canal. One can isolate and cut this mandibular lateral-line branch without much difficulty, leaving a pure preparation of ampullary end-organs.

The electrical apparatus employed consisted of a five-stage condenser-coupled amplifier, Matthews oscillograph, moving-film camera and loudspeaker.

After pithing the ray, the nerve was dissected out and cut centrally, leaving a length of 2 or 3 cm. in connexion with the ampullae. This peripheral length of nerve was slung on platinum wire electrodes, the earth electrode being nearest the ampullae. Recording was monophasic, and afferent action potentials appeared on the records as upward displacements of the base-line.

Principal experimental facts. The main experimental results emerged with complete clarity from the very beginning of the investigation, and were confirmed without any exceptions on over forty preparations. These results were so unforeseen and altogether so extraordinary that it would be as well to enumerate them briefly at this point before proceeding to a discussion of the detailed evidence.

The activity of the ampullae of Lorenzini is defined as follows:

(1) Like lateral-line receptors they display an autonomous rhythm of activity.

(2) They have no mechanical sensitivity whatever.

(3) They are exceedingly sensitive to temperature.

(4) Their response to *cooling* is an acceleration, their response to *warming* is a retardation or inhibition of the rhythm of impulse discharge.

Qualitative experiments. Recording from the whole nerve one observes a massive asynchronous discharge in which it is impossible to identify the individual action potentials. The whole nerve, as I have described, contains several hundreds of fibres supplying the mandibular ampullae as well as a smaller bundle innervating the mandibular lateral-line canal. Thus the preparation at this stage contains both lateral-line and ampullary end-organs, and displays that extreme vibrational sensitivity so characteristic of active lateral-line receptors. Gentle tapping on the table on which the preparation is set up is adequate to augment and accelerate the asynchronous oscillations. And the response to direct mechanical stimulation of the animal's skin is even more violent. These responses are completely abolished when the lateral-line branch of the nerve is cut. The asynchronous spontaneous discharge, now confined to the ampullary fibres, goes on as before, but no change in its volume can be detected when mechanical stimuli are applied. The complete absence of mechanical sensitivity is further confirmed when, after section of the lateral-line branch, the nerve bundles supplying the mandibular ampullae are also separated out and all except one are cut. One is left then with a preparation in which the number of active fibres has been sufficiently reduced to permit the recognition of individual impulse trains in the records and also in the sound from the loud-speaker. No kind of mechanical stimulation, such as touch, stroking or pressure, has any effect on the frequency of these discharges.

The response to temperature was first discovered when elasmobranch

Ringer which had been cooled or warmed a few degrees below or above room temperature was dropped from a pipette on to the skin overlying the mandibular ampullae. A drop or two of cooled Ringer evoked an immediate acceleration, the frequencies of individual rhythms rising abruptly to something of the order of 70–100 per sec., and producing in the loudspeaker a clear musical note, whose pitch, after reaching its peak, declined gradually over a period of several seconds until finally the original frequency (8–16 per sec.) was re-established. Conversely, a drop or two of warmed Ringer caused an immediate cessation of the discharge, even in preparations containing a large number of active units. After a total inhibition lasting several seconds, one unit would begin discharging, then presently another, at first slowly, then more rapidly, until before long the original rhythm had again returned. Such responses could be repeated over and over again.

The response to warming could also be evoked by means of a hot glass rod, held about 1 cm. above the mandibular ampullae. When the glass rod was brought near the discharge ceased, when the rod was withdrawn it gradually started up again. Applied to the back of my hand at a similar distance, the rod produced a mild sensation of warmth.

Isolated preparation and temperature chamber. The mandibular ampullae are contained in a smooth membranous envelope which has very loose attachments with the surrounding tissues, so that the whole mandibular capsule with its nerve could be cleanly removed, thus providing a neat and small isolated preparation, which was capable of maintaining its activity for hours. Fig. 2 (Plate 33) is a photograph of the preparation, showing the mandibular capsule of a small ray with its brush of ampullary tubes cut across, its nerve, and the mandibular lateral-line branch by-passing the capsule. A few ampullae are dimly discernible by transparency inside the capsule. The nerve has been cut down so as to leave only a few intact fibres. The scale is 5 mm.

This preparation served for the quantitative study of the responses of individual ampullary end-organs to temperature changes. It was installed in an apparatus which is illustrated in fig. 3. The mandibular capsule is laid in a little puddle of 2 or 3 drops of elasmobranch Ringer in the depression in the glass bulb *B*, and the nerve is slung on the platinum wire electrodes *P*. Ice-water *C* and warm water *W* are delivered through the two-way tap to the bulb and escape at the outflow *O*. This glassware is mounted in a brass pot with a flanged, tight-fitting lid. The pot is filled with paraffin wax up to the level of the bulb. The wax serves as a support for the bulb and platinum wires, and also reduces the air space in the chamber, thus facilitating the maintenance of a saturated atmosphere inside, which is further assisted by a piece of moist blotting paper adhering to the under surface of the lid. The temperature of the water in the bulb is recorded by the thermometer *T* which is fixed to lie horizontally within it, and whose stem projects outside the pot. When warm or cold water is made to flow through the bulb the change in temperature of the mandibular capsule lying on the surface of the glass is recorded with sufficient approximation by the response of the thermometer. Owing to differences in thermal conductivity the warming or cooling of the tissue would take somewhat longer than the change registered by the thermometer, but such differences have little significance at the level of accuracy that is required in these experiments. The thermometer readings were certainly an adequate measure of the temperature at the surface of contact between the mandibular capsule and the glass, and this, from the point of view of sensory function, is the variable that interests us most. With care one could manipulate the tap so as to adjust the thermometer reading to any desired

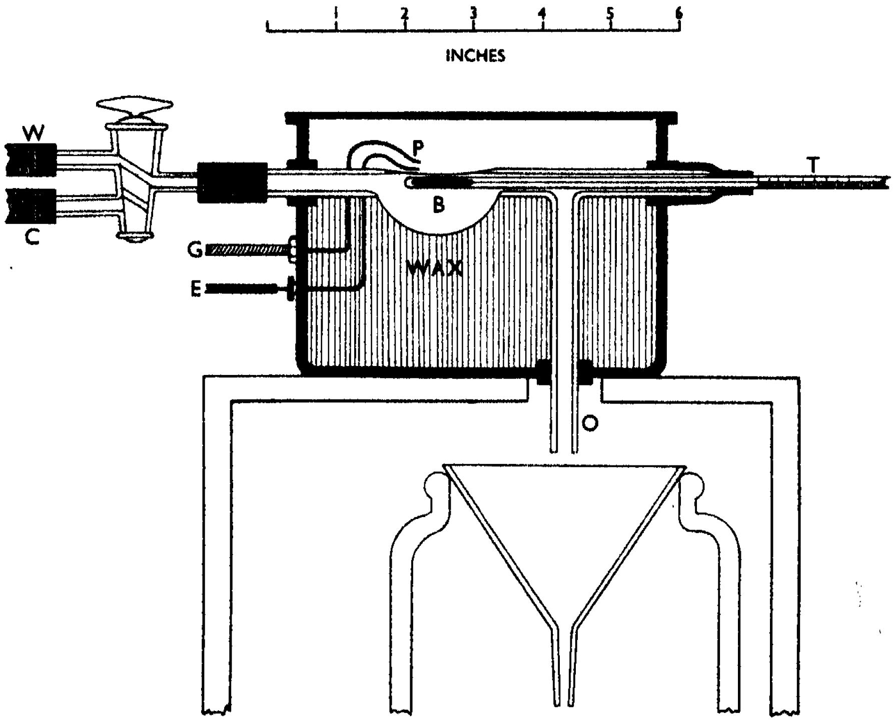


FIG. 3. The temperature chamber. Explanation in text.

value, and one could keep the temperature constant within ± 0·1° C. for any length of time. A mechanical device was employed to signal the two-way operations of the tap, so that the beginning and end of a rise or drop in temperature was marked on the record. These signals were accurate to within 1 sec., and served admirably to identify a long series of random temperature changes.

A preparation installed in this apparatus will remain active for several hours. Two of my preparations continued overnight and were still discharging rhythmically 24 hr. after being set up. The autonomous frequency declines very slowly, while the sensitivity to temperature declines at first rather steeply, but presently attains a steadier level. It was a common experience to find that when a preparation was first set up a change in temperature of 0·1° C. in either direction was adequate to evoke a marked response, but about half an hour later a difference of about 1° was required to produce a comparable effect. This order of sensitivity may then be maintained with little change for several hours, though eventually the sensitivity to temperature changes becomes weak. The responses of the two preparations that had survived overnight were feeble, though their autonomous rhythms were sufficiently vigorous.

Response of single end-organs to temperature changes. The general character of the responses recorded is shown in the accompanying records which were selected from a large number of similar records taken from thirty preparations of the isolated mandibular capsule of *Raja*. In all the oscillographic records the time signal is in seconds. The white line below the base line is the temperature signal. Its downward displacement marks a flow of warm water, its upward displacement marks a flow of cold water through the apparatus. The initial and final temperatures of each temperature change were noted and are shown on the records. Where several lengths of record are shown to illustrate a single response, the seconds following the establishment of a new temperature are consecutively numbered. Artefacts caused by the manipulation of the tap are marked with a ×.

Fig. 4 (Plate 34) is from preparation No. 24 from a small *Raja maculata*. The records were taken about 1½ hr. after the animal was killed by pithing. They show a response to a rise in temperature of 0·3° (records A and B, continuous), and a response to a drop in temperature of 0·7° (records C and D, continuous). A single rhythm of large potentials is clearly seen, although at least one other train of small potentials is present. When these small potentials coincide with one of the large potentials, they give rise to one of the extra-large potentials occurring irregularly in records C and D. The rise in temperature from 14·0 to 14·3° causes a retardation in the rhythm extending over several seconds (record B). The drop from 14·0 to 13·3° causes a prolonged acceleration (records C and D).

Fig. 5 (Plate 34) is from a more complex preparation (No. 13). There are several rhythmically active units. The retardation resulting from a rise in temperature from 15·4 to 16·0° is striking (records A and B continuous), and so is the acceleration following the reverse change from 16·0 to 15·3° (record C). In record D a rise from 10 to 12° causes complete inhibition. This figure illustrates a fact which has been abundantly confirmed in numerous experiments, namely, that the behaviour of all the ampullary receptors is the same.

In the experiments of fig. 6 (Plate 35) the recording was continued long enough to show the adaptation of the end-organ to the new temperature. Records A–D show the prolonged response to a drop of 0·4° (14·0 to 13·6), and records E–H show the total inhibition lasting about 25 sec. after a rise of 1°.

These relations hold throughout the physiological temperature range. I have not recorded at temperatures below 3° C., and above about 20° the receptor mechanism suffers irreversible damage, and the rhythm of discharge usually soon stops. But within these limits the acceleration in response to a fall in temperature and the inhibition in response to a rise can always be demonstrated, as fig. 7 (Plate 35) shows. Record A is a cooling response in the lower temperature range. Following this response the temperature was raised from 7 to 13° when there was a period of inhibition lasting 45 sec. The temperature was maintained at 13° for 5 min., during which time a discharge of uniform frequency continued. The temperature was then raised further from 13 to 18°. There was an immediate cessation of the discharge, and this time the inhibition was apparently permanent, for the preparation remained silent for 25 min., during which time the temperature was maintained at 17·5–18°. Then the temperature was dropped to 15°, and this cooling immediately revived the preparation. The response is shown in fig. 7 (Plate 35), records B, C and D. The temperature was then raised again to 18°, but this time in three steps of 1° each. This gradual warming produced only a temporary arrest at each step, and at 18° the preparation was firing vigorously. Record E, fig. 7, begins after 2 min. at 18°, and the frequency is 17 per sec. Rapid cooling down to 15·5° (records E, F and G) evoked a response almost identical with the preceding one.

All these records will suffice to illustrate the quality of the experimental results obtained in this investigation. They show that there was ample material for the counting of the frequencies of discharge from individual receptor units. For purposes of quantitative comparison the curves obtained when individual frequencies are plotted against time are far more illuminating than short lengths of oscillographic records. In the following figures the main characteristics of the temperature response are presented in this fashion. In fig. 8 are shown four responses to temperature changes of less than 0·5°. Two are warmings and two are coolings. The vertical line at 4 sec. marks the beginning of the change in temperature. The initial and

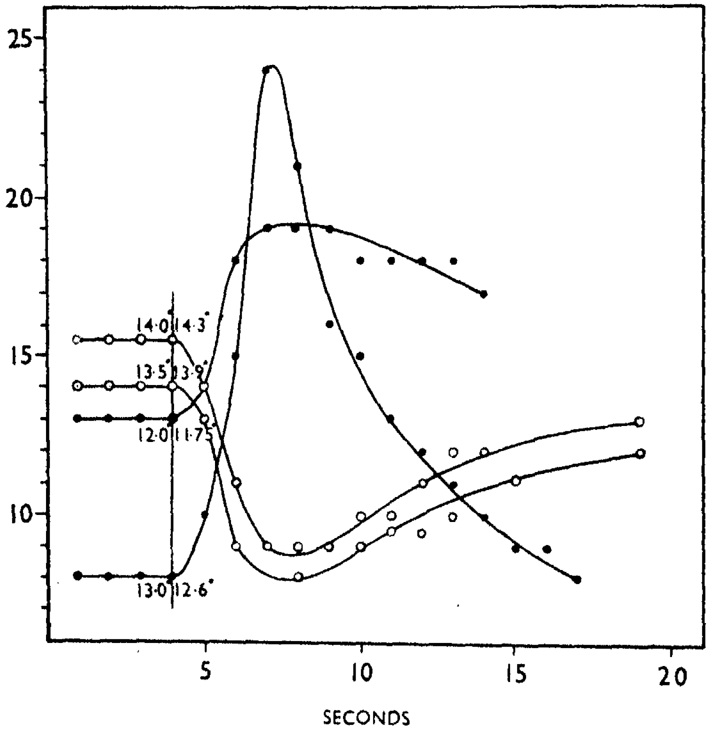


FIG. 8. Responses of single units to temperature changes of less than 0·5° C.

final temperatures for each curve are marked on either side of this line, though the final temperature is attained only at a point 3–5 sec. to the right of the line. In fig. 9 four responses are similarly shown for temperature differences of 1·0–2·0°. Figs. 8 and 9 indicate the order of sensitivity of the ampullae of Lorenzini to temperature changes, and they also serve to emphasize the proportionality between the degree of acceleration or inhibition and the magnitude of the temperature change. It will be noted that in fig. 9 both warmings cause total inhibition, while in fig. 8 the two inhibitions are partial. Similarly, in fig. 9 the accelerations caused by cooling attain frequencies which are about double the frequencies attained in fig. 8. This intensity-frequency relationship is further illustrated in fig. 10, which shows a drop of 0·5° (16·5 to 16·0°) followed after 1 min. by a further drop of 2·0° (16·0 to 14·0°). The variation in temperature is indicated by the line at the top of the graph. Although the general proportionality of the intensity-frequency relation is clear enough, the plotting of an intensity-frequency curve was hardly feasible, because the sensitivity of the isolated preparation does not remain absolutely constant for a sufficiently long time. Fig. 10 indicates also a second proportionality, namely that between the magnitude of the stimulus and the adaptation time. In the first response a steady frequency is attained within about 10 sec. after the temperature change; in the second response it takes about

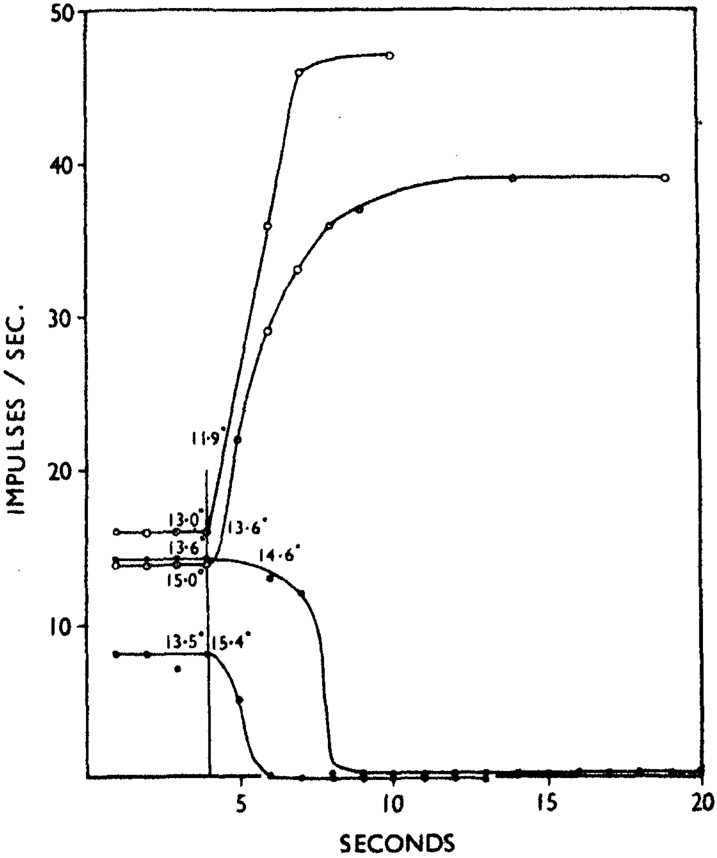


Fig. 9. Responses of single units to temperature changes of 1–2°.

a minute. Even longer adaptation times (2–3 min.) have been recorded for large temperature changes. These are no doubt partly accounted for by differences in the time of *thermal* equilibration, though it is unlikely that this is the major factor, for the peak frequency is always rapidly attained whether the temperature difference be small or large.

Fig. 11 shows four responses at the extremes of the temperature range. There is a warming and a cooling in the region 5–8° and a warming and a cooling in the region 18–20°. This figure illustrates what is perhaps the most astonishing aspect of this very remarkable mechanism. The statement that cooling causes acceleration and warming causes inhibition undoubtedly holds for any temperature at which the rhythmical mechanism can continue to function. In the whole series of experiments there was not the slightest indication of an optimum temperature such that a deviation from it either up or down the temperature scale would result in a reduction in frequency, and an approach towards it from either end would cause an acceleration. (This will be made still clearer when we come to consider the steady, adapted frequencies in function of temperature.) But this statement is not intended to imply that the sensitivity of the receptor is the same at all temperatures. The responses shown in fig. 11 are the best that have been recorded at the extremes of the temperature range. My whole experience with these preparations has given me a strong impression that the sensitivity is greatest in the region 10–15°. It is difficult to be certain on this point because the extreme sensitivity of a fresh preparation must always be first tested at room temperature, and because the drastic temperature change required to transfer the preparation from room temperature to either extreme of the temperature range appears to have a markedly deleterious effect on the sensitivity.

Stable frequency-temperature relation. After the response to a temperature change has taken place adaptation occurs, and the frequency of discharge returns again to the initial value, as, for example, in fig. 10. This, at least, appears to be what happens when the temperature changes are of the magnitude that we have hitherto considered, i.e. 0·5–2°. When, however, the effects of larger temperature differences are examined, it is found that the frequency at the end of the response is not quite the same as it was at the beginning. After a rise in temperature of several degrees the rhythm, having recovered from a period of inhibition, gradually accelerates until it attains a steady frequency which is higher than the frequency before the response, and after a drop of several degrees and the resulting maximal acceleration, the rhythm adapts to a lower frequency than before the response. This is seen to be so in fig. 12, which illustrates a 20 min. experi-

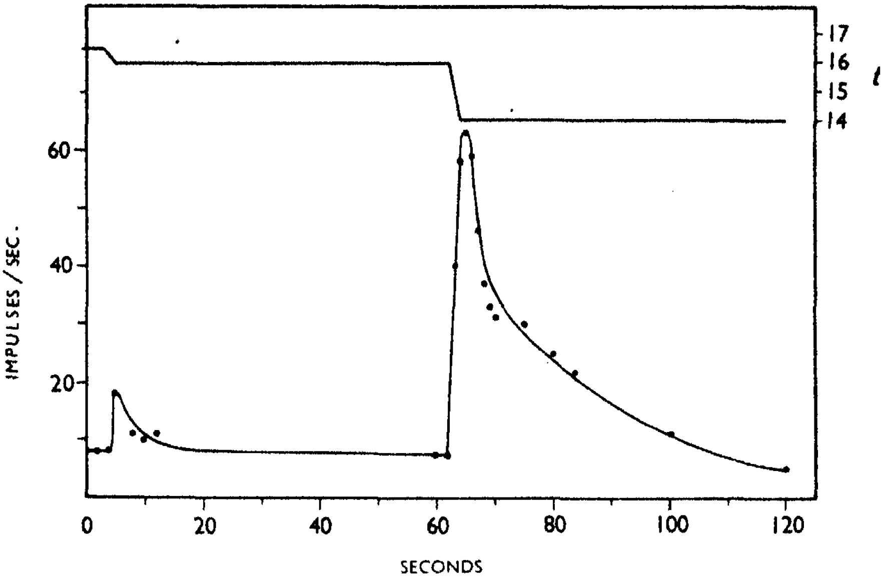


FIG. 10. Response to a drop of 0·5°, followed by a drop of 2°.

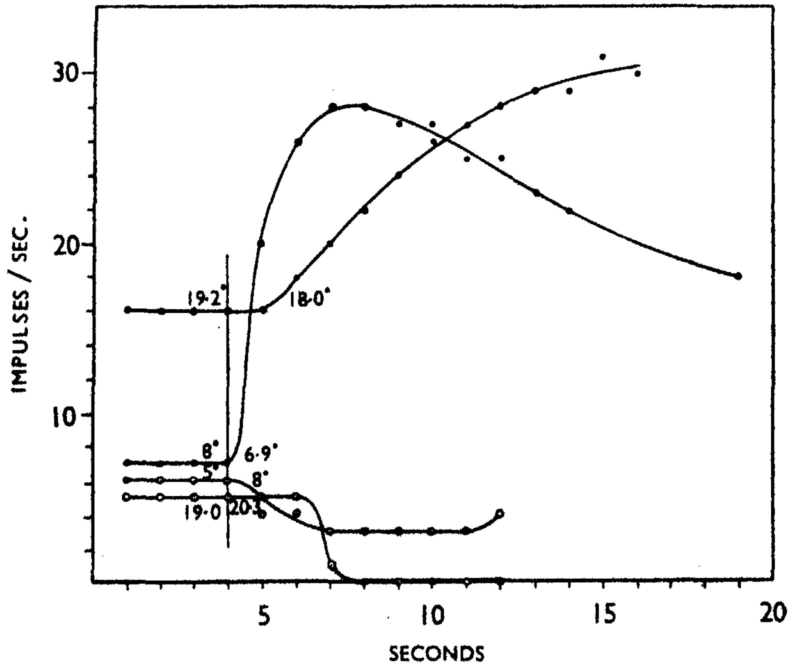


FIG. 11. Responses to warming and cooling at the extremes of the temperature range.

ment in which the temperature was raised from 10 to 15°, maintained at 15° for 10 min., then lowered again to 10°, and maintained at 10° for another 10 min. The adapted frequency was 7 per sec. at the beginning. After the first response it rose to 11 per sec., and after the second response it sank again to 8 per sec. It is clear, therefore, that when we consider conditions of stable equilibrium, the frequency of discharge varies as a function of temperature, and displays a positive temperature coefficient. In this important respect our paradoxical system behaves in an orthodox manner, indeed it behaves like every other biological rhythm that has ever been studied. An experiment such as that of fig. 12 at once suggests the possibility of deriving a temperature curve for this sensory rhythm, and determining its temperature characteristics. In the oscillographic records of fig. 13 (Plate 36) the increased frequencies of two units in function of temperature are shown between 9 and 18° C. Similar records were taken at different temperatures from three preparations. In all cases the equilibration time allowed at each temperature was 5 min., which was certainly sufficient for the system to attain a stable equilibrium, for when, on several

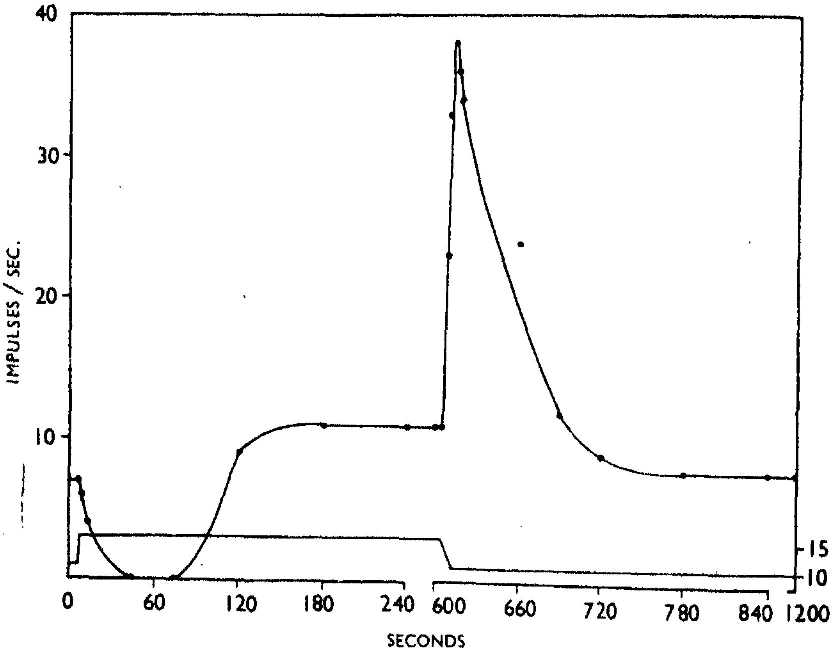


Fig. 12. Temperature raised from 10 to 15°, and, after 10 min., lowered again to 10°.

occasions, the temperature was maintained constant for 10 min. no change in frequency of discharge was observed. The values recovered from one such experiment (preparation no. 24) are given in fig. 14. The preparation had been installed at 12.30 p.m., and these determinations were made between 3 and 4.30 p.m. The points are numbered in the order in which they occurred in the series. It must be borne in mind that each change from one temperature to another was accompanied by the usual response, inhibition or high-frequency discharge, depending on the direction of the temperature change. Although it is quite clear from fig. 14 that the autonomous rhythm of the ampullary end-organs possesses a positive temperature coefficient, the results are disappointing inasmuch as it is not possible to derive an exact temperature-frequency relationship. The four last determinations (nos. 10–13) fall on a straight line, and these give $Q_{10} = 1{\cdot}9$ between 5 and 15°.

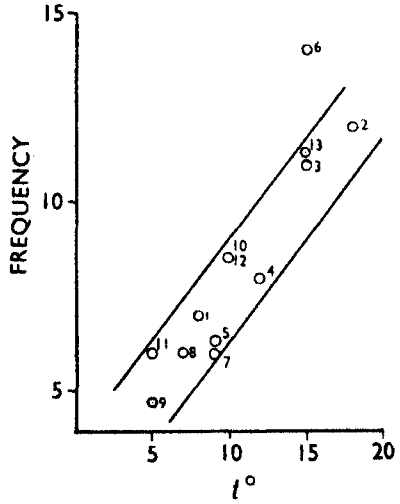


FIG. 14. Stable frequencies of a single unit of ampullae of Lorenzini in function of temperature.

In fig. 15 are plotted the values derived from preparation no. 9 (squares, numbered in serial order) and two consecutive series from preparation no. 7. This preparation was installed at 1.30 p.m. The determinations plotted as black circles were begun at 3 p.m., and the series plotted as white circles was begun at 4 p.m. Again it is possible to draw straight lines through some of the points. For preparation no. 9 $Q_{10} = 2{\cdot}4$, for preparation no. 7 (black circle series) $Q_{10} = 2{\cdot}3$.

The variability of the data is probably due in part to the relative crudity of the technique, though I think it is mainly to be ascribed to an inherent instability of the preparations. It is possible that more uniform results would be obtained with the organs *in situ* in the whole animal, with intact circulation, and under artificial respiration. This, of course, would require a totally different technique.

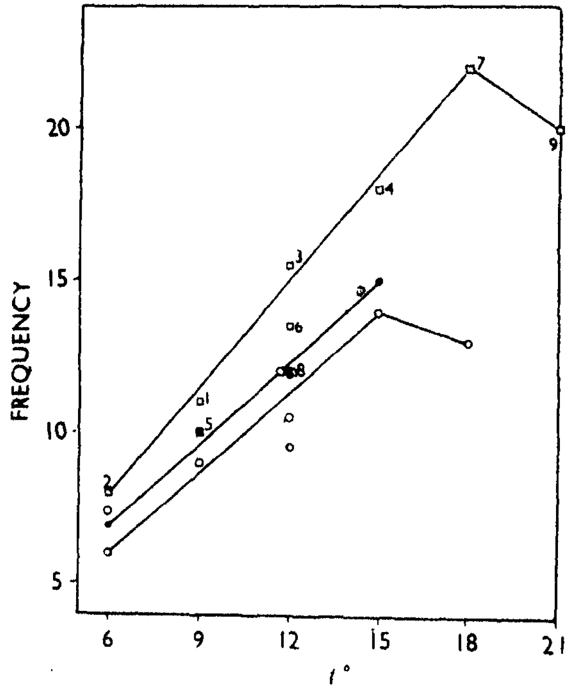


FIG. 15. Stable frequencies of two preparations of ampullae of Lorenzini in function of temperature.

Synchronization and abnormalities. In preparations containing a large number of active fibres the discharge is always asynchronous, and when the number of fibres is sufficiently reduced to permit the identification of individual impulse trains, it is seen that, although the several rhythms usually have very similar frequencies, they are always independent of one another. Fig. 13 (Plate 36) illustrates this. When, as a result of a considerable drop in temperature a high frequency of discharge is evoked there is still no evidence of synchronization. The case of preparation no. 17 is of interest in this connexion. When equilibrated at 18° there was a single rhythm of 13 impulses per sec. The preparation was cooled down to 8°

(fig. 16, Plate 36); the rhythm accelerated to 88 per sec. and at the same time another rhythm of small potentials appeared and accelerated. In fig. 16, record B is the continuation of record A, which begins just after the start of the cooling. The two high-frequency discharges together produce in record B a regular undulation in the magnitude of the potentials, which I interpret as a beat-frequency effect resulting from the superposition of two independent rhythms of different frequency.

In three preparations there appeared, 2 or 3 hr. after their installation, a phenomenon which was probably abnormal in the sense that its occurrence is confined to ampullary organs whose physiological condition has undergone considerable deterioration. The phenomenon consisted in the appearance of regularly recurring bursts of high-frequency discharge with periods of quiescence in between. Two such effects are shown in fig. 17 (Plate 36). Both are from preparation no. 13, which, when installed at 3 p.m., responded normally to variations in temperature. The preparation was complex, containing several active fibres. After 15 various warmings and coolings, its sensitivity had declined considerably, and then, at 4.40 p.m. it began to behave as shown in record A of fig. 17. The bursts of discharge clearly come from a single fibre, though some random impulses from other units appear to be included in them. Cooling the preparation through 10° excited a number of other units, and an asynchronous discharge occurred (record B). The bursts, apparently, had ceased. After that, the discharge remained continuously rhythmical at various temperatures for nearly an hour; then it began to fail. A drop in temperature of several degrees would evoke a discharge which ceased after 20 or 30 sec. Then at 5.40 p.m. the bursts appeared again. The preparation was at 18°, and this time the bursts were very brief, consisting of half a dozen impulses or so in rapid succession. with long intervals of complete quiescence in between. A portion of this discharge is shown in record C of fig. 17 (Plate 36). The bursts occur with a remarkably regular slow rhythm, the period being 1·2 sec. Moreover, it is clear that at least two, possibly more, units contribute to each burst. This was the only occasion on which evidence of synchronization was encountered.

In another preparation, no. 8, the abnormal behaviour was somewhat different in form. It is illustrated in fig. 18 (Plate 36). Each grouping occupied about 2 sec., and was composed of 9 impulses accelerating slightly, and terminating in a rapid volley of about 50 impulses in half a sec. At the same time another unit was firing regularly at 16 per sec. (smaller potentials). Here there was no synchronization. The complex groupings of large potentials continued for about 45 min., changing progressively in form. Records A and B of fig. 18 show two consecutive groupings as they first appeared. They are almost identical, and were separated by a period of 8 sec. Gradually the intervals between the high-frequency volleys lengthened, while at the same time the duration of the slow rhythm leading up to each volley increased, thus reducing the interval of silence following each of the volleys. Record C of fig. 18 shows the discharge 15 min. after record B. The interval between successive volleys had now become 50 sec., while the slow rhythm was almost continuous. The bursts continued for another half hour, the intervals between the last three were 1 min. 15 sec., 1 min. 45 sec., and 6 min. 30 sec. After that the rhythm of large potentials became continuous and there were no more volleys of high-frequency discharge.

These phenomena seem to me of peculiar interest, because they show that the mechanism is capable of exceedingly slow, yet perfectly regular rhythms of activity, which, so far as can be judged, play no part in its normal behaviour. They show also that the rhythmicity of the system is not confined to the production of regular trains of impulses, but that rhythms of a second and third order can arise to create highly complex but still regular groupings.

Effects of temperature changes on lateral-line and stretch receptors. When working with lateral-line organs I had found that small portions of a lateral-line canal could be excised together with a little of the surrounding muscle and skin, and would continue to discharge impulses for some time. The autonomous rhythm of such excised lateral-line preparations was not nearly so persistent as that of the isolated preparation of mandibular ampullae; in general the lateral-line system seems to be less resistant to surgical manipulation than the ampullae of Lorenzini. I now set out to test such excised preparations of the hyomandibular lateral-line canal of *Raja* in the same temperature chamber, the expectation being that they would show no response to temperature changes other than the usual frequency-temperature relationship of rhythmical systems. To my complete surprise I found that lateral-line receptors displayed the same paradoxical behaviour with respect to temperature changes as the ampullae of Lorenzini, *but with an entirely different order of magnitude.* The rhythm of impulse discharge was inhibited by a rise in temperature and accelerated by a drop in temperature, but in order to induce the effect at all it was necessary to employ temperature differences of the order of 10°, and even then the response was insignificant compared with the responses of the ampullary organs.

The state of the preparation could always be tested by its sensitivity to

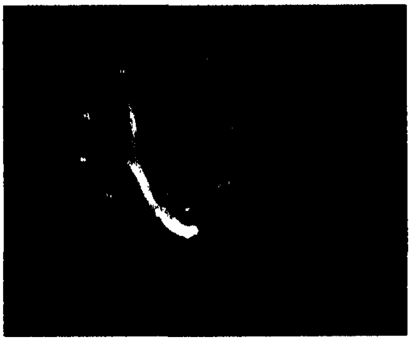


FIG. 1. A few of the hyomandibular ampullae of *Raja*. Scale 5 mm.

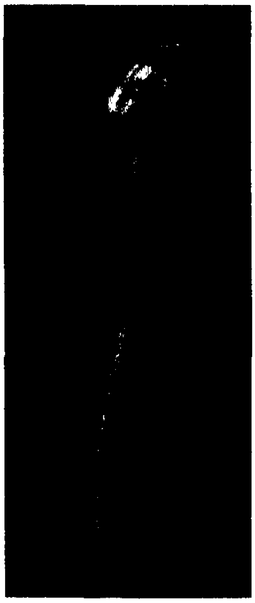


FIG. 2. Isolated preparation of the mandibular capsule. The nerve has been reduced to a few fibres. Ampullary canals cut across. Lateral-line branch can be seen by-passing the capsule. Scale 5 mm.

FIGS. 4, 5, 6, 7, 13, 16, 17 and 18. In all oscillographic records the time signal is in seconds. The white line below the base line marks changes in temperature. Its upward displacement signals a flow of cold water, its downward displacement a flow of warm water through the temperature chamber. Artefacts marked ×.

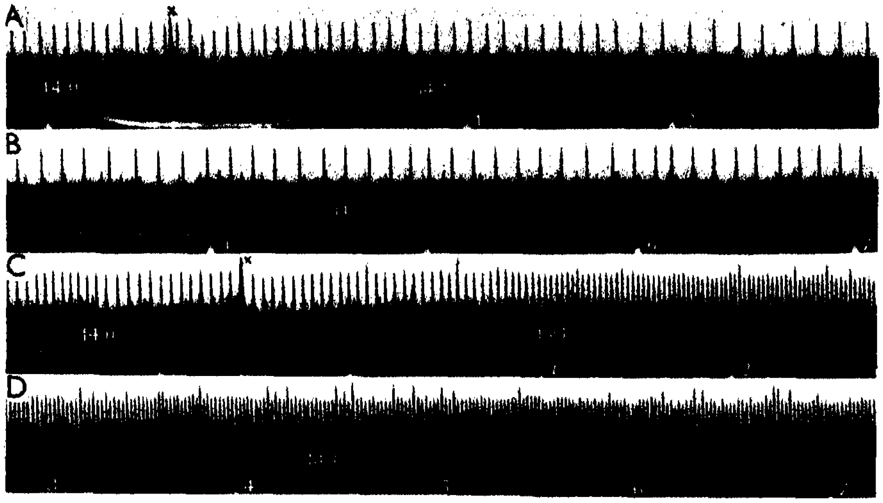


FIG. 4

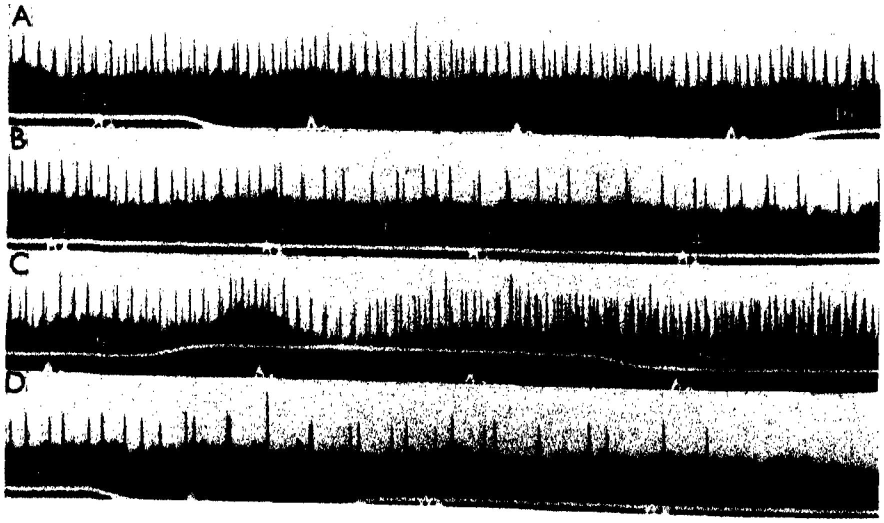
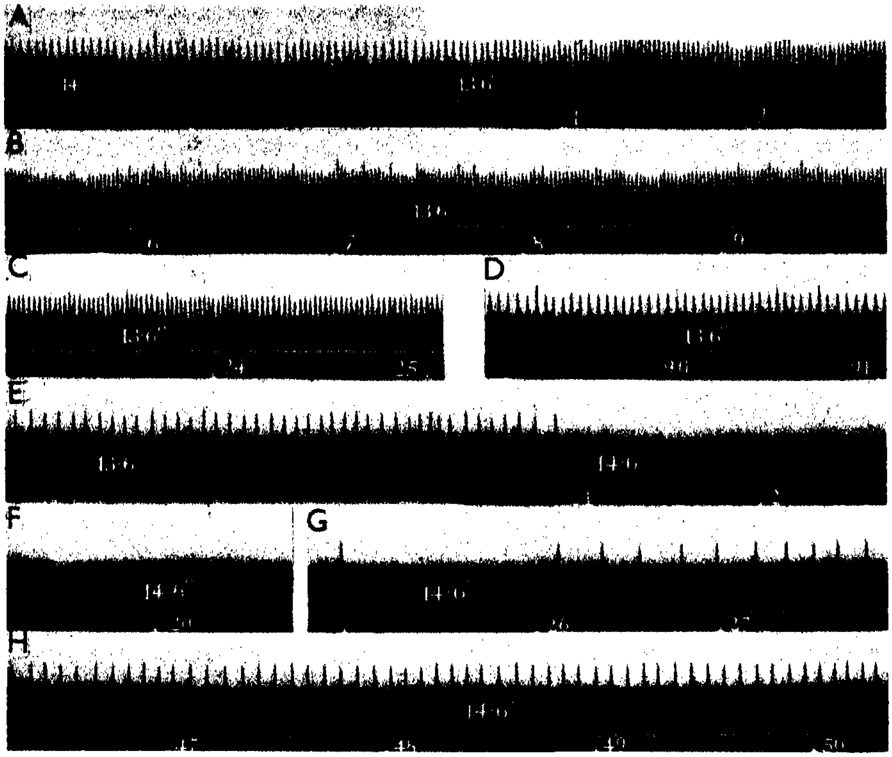


FIG. 5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13·6
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Fig. 6

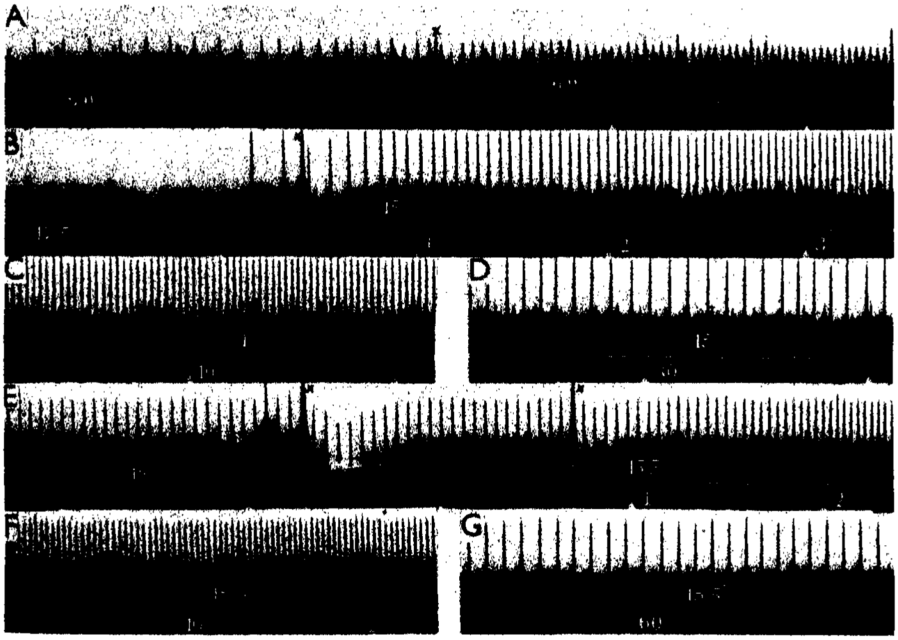
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Fig. 7

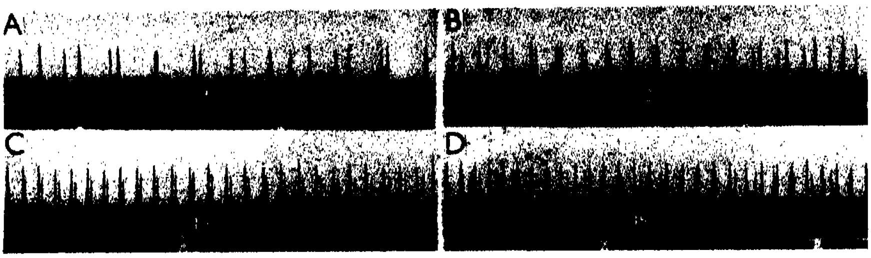

Fig. 13

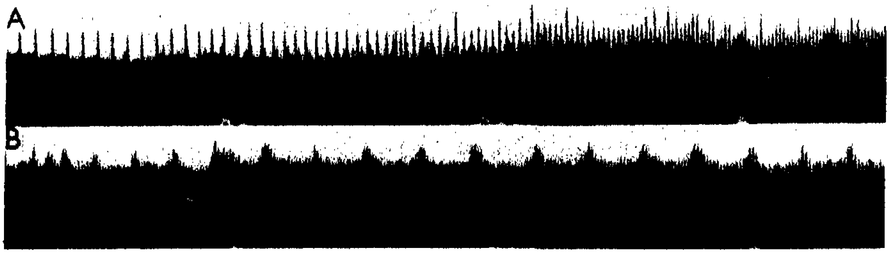

Fig. 16

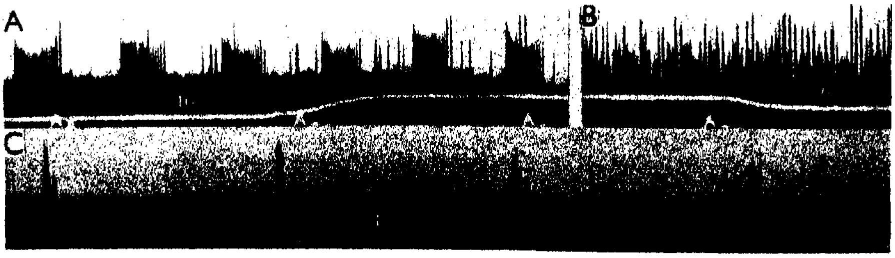

Fig. 17

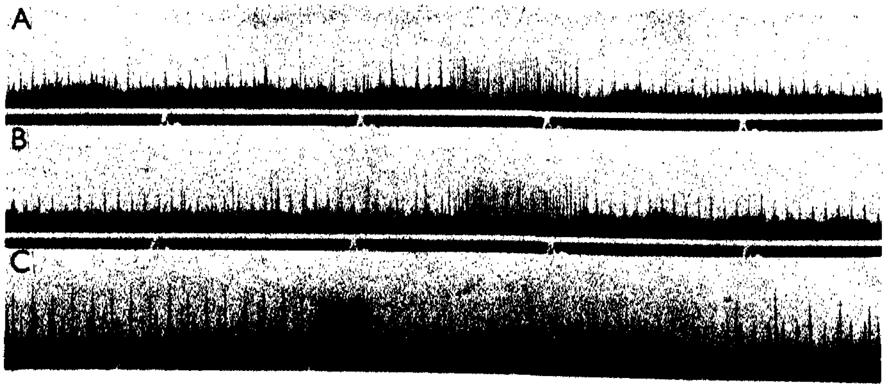

Fig. 18

vibration, and when this was delicate it was a clear sign that the receptor mechanism was in a satisfactory condition. With numerous preparations whose vibrational sensitivity was normal the result was always the same. The paradoxical temperature response was there, but it was very weak. Fig. 19 shows the strongest response recorded. Warming the preparation from 7 to 17° produced an inhibition lasting about 10 sec., and cooling down to 7° again caused an acceleration of only 30 %. This is to be compared with accelerations of 500–1000 % frequently observed with ampullae of Lorenzini cooled through 1 or 2° C.

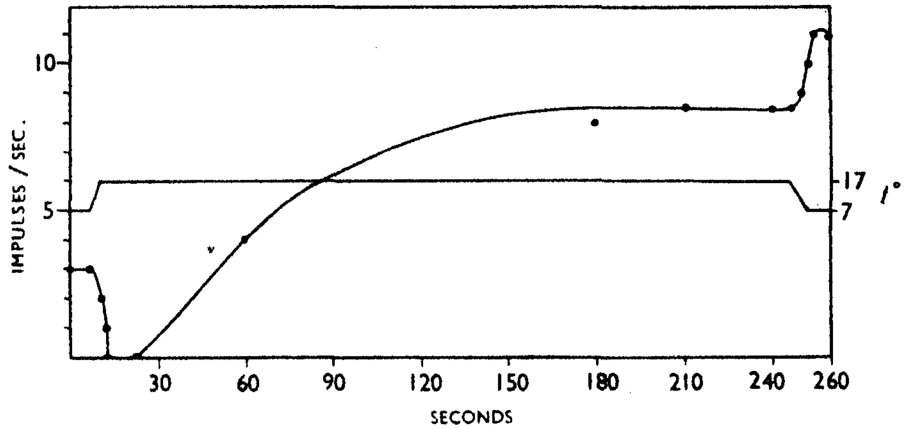

FIG. 19. Responses of lateral-line receptor to temperature changes of 10°.

Attempts to determine equilibrated frequencies at different temperatures were less successful than with the ampullae of Lorenzini, because in most cases the autonomous rhythm did not persist for a sufficient time. The data from two preparations are shown in fig. 20. The lines drawn through the points are not intended to imply a linear relation. Exponential curves could equally well be fitted. The Q_{10} values of the two sets of data are not consistent. In one $Q_{10} = 1{\cdot}4$, in the other about 3·0. The curves show no more than that the autonomous rhythm of the lateral-line receptors has a positive temperature coefficient.

What has been said concerning the temperature responses of lateral-line receptors applies also to stretch receptors in the muscles of the ray. The preparation used was a modification of the one previously employed by Fessard and Sand (1937). It was an isolated nerve-muscle preparation of the anterior flexor muscle of the pelvic fin. This muscle, derived from very young rays, was less than 1 cm. in length. It was laid on the surface of the glass bulb in the temperature chamber, with the nerve slung on the electrodes, and was secured in a slightly stretched position with two pieces of plasticine. There do not appear to be more than two or three receptors in this muscle, and it is easy to obtain single end-organ preparations. The sensitivity of the receptor could be tested by gently stretching the muscle. Under slight tension the receptor usually settled down to a slow steady rhythm of discharge, whose duration, in different preparations, was very

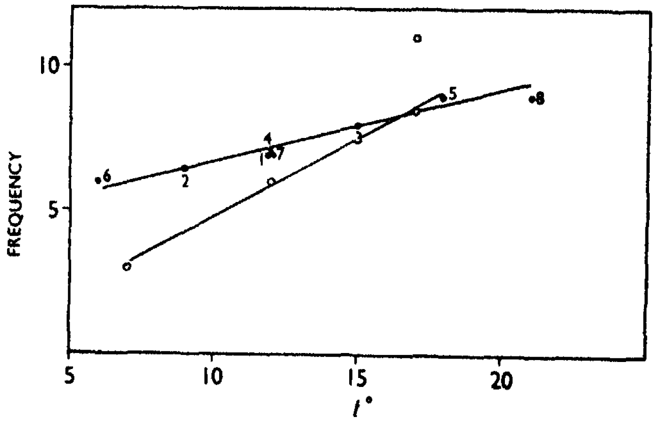

Fig. 20. Stable frequencies of two lateral-line receptors in function of temperature.

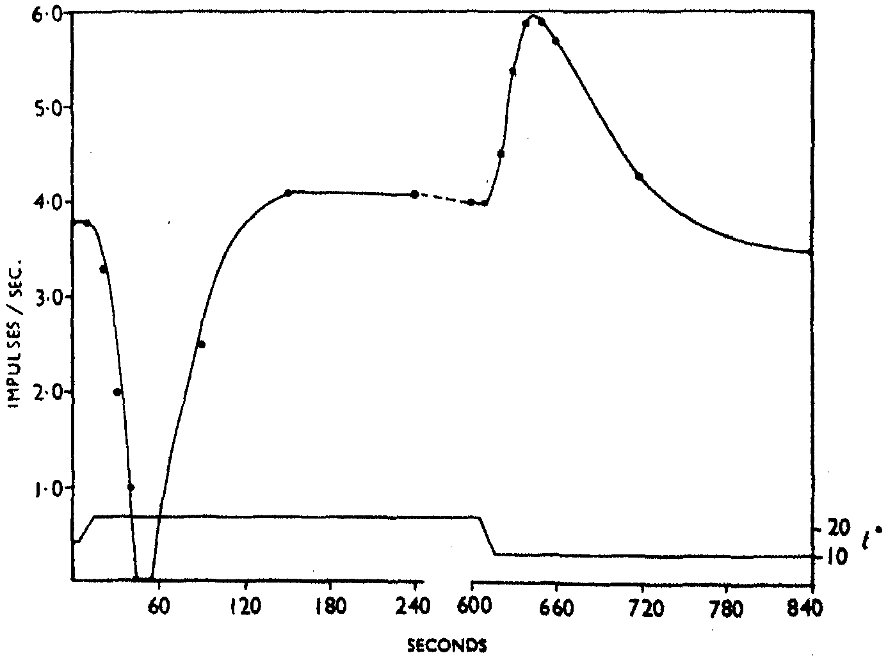

Fig. 21. Responses of stretch receptor to temperature changes of 10°.

variable. In most, the discharge continued only for 5–15 min. and then, in the manner so characteristic of these organs, stopped abruptly without preliminary retardation. A slight increase in tension would then start up a fresh discharge. In some preparations, however, the receptor continued to discharge a regular rhythm for an hour, or even longer. These were used to test the effects of variations in temperature. In no instance did small temperature changes of 1–2° produce any noticeable effect on the rhythm, but large changes of 10° or so evoked retardation or acceleration to an extent closely similar to the same phenomena in lateral-line receptors. One such experiment is illustrated in fig. 21. It does not require further description.

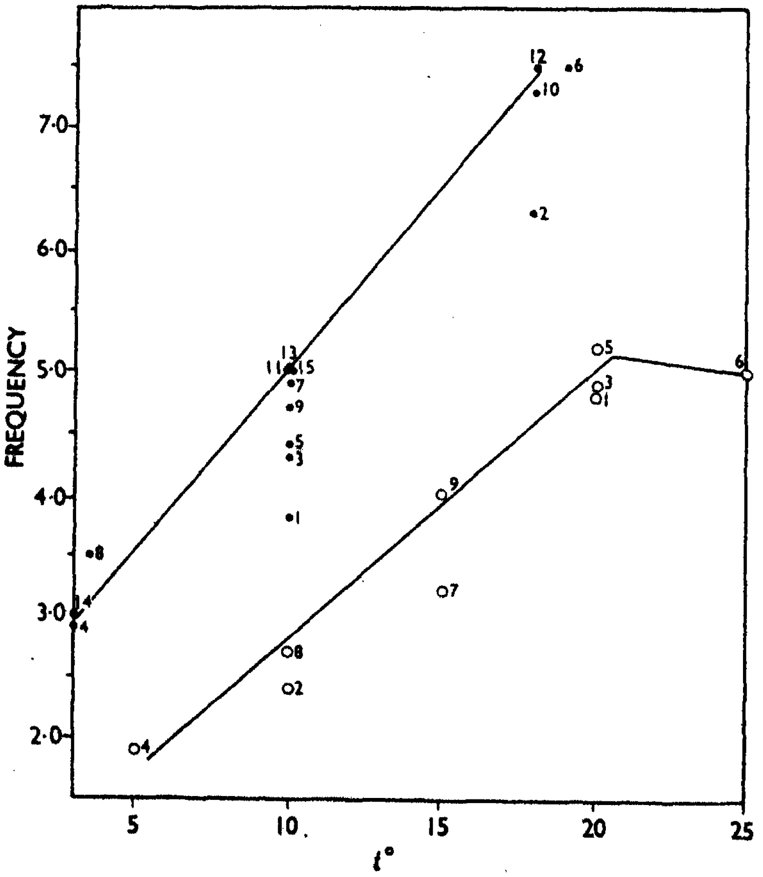

FIG. 22. Stable frequencies of two stretch receptors in function of temperature.

Equilibrated frequencies at different temperatures derived from two preparations are plotted in fig. 22. Throughout the whole series of determinations each muscle remained, of course, under constant tension. The fifteen determinations plotted as black circles occupied about 80 min. It will be noted that, as judged by the frequencies at 10°, the black-circle series shows a regular acceleration with time, for which I am unable to suggest an explanation. The white-circle series shows this also for the three values at 20°, though determination no. 6, whose value indicates the harmful effect of so high a temperature as 25°, evidently served to depress the subsequent frequencies at lower temperatures. The last five determinations of the black-circle series (nos. 11–15) show that the preparation had reached a state of complete stability, and these five points fall very accurately on a straight line. Between 5 and 15° $Q_{10} = 1{\cdot}9$ for the upper curve and 2·1 for the lower.

DISCUSSION

Nature of the mechanism. If the evidence for the paradoxical effects of temperature changes on the rhythm of impulse discharge from the ampullae of Lorenzini were less conclusive, one would say that such a phenomenon is impossible. But in face of the overwhelming evidence one is constrained to cast about in one's mind for an explanation. A real explanation, in the experimental sense, is not, for the time being, to be expected. All that can be done with the material at one's disposal is to suggest a basis for a working hypothesis which would raise the phenomenon from the level of extreme improbability and place it among facts awaiting further experimental analysis.

Our conceptions concerning the effects of temperature on biological processes are based, in the first instance, on the experience of chemistry. But, as Bělehrádek (1935) has emphasized, many biological processes obey very imperfectly the quantitative laws which have been derived from the study of chemical reactions, as witness, for example, the inconstancy of Q_{10} at different temperatures. Such inconstancies are due to the physico-chemical complexity of even the simplest biological systems, and it is not inconceivable that the interrelationship of the component reactions of a given biological system may be such as to give rise to a form of behaviour with respect to temperature which appears to flout the fundamental laws of chemical action.

On the assumption that the paradoxical temperature effect is due to ascertainable physico-chemical causes, the following scheme is offered as a first approximation to a working hypothesis. It is assumed that the frequency of impulse discharge is a function of the difference between the velocities of two processes. It is further assumed that one of these processes, *I*, when subjected to a change in temperature, attains a new equilibrium rapidly, whereas the other process, *E*, attains a new equilibrium slowly. The consequences of these assumptions are illustrated by fig. 23. To give the illustration a concrete form the curves have been derived from arbitrary values such that the velocity of process *E* is taken to be 4·0 at 0°, and its Q_{10} as 2·5 at all temperatures; and the velocity of process *I* is also 4·0 at 0°, and its Q_{10} 2·0 at all temperatures. The curves *E* and *I* then

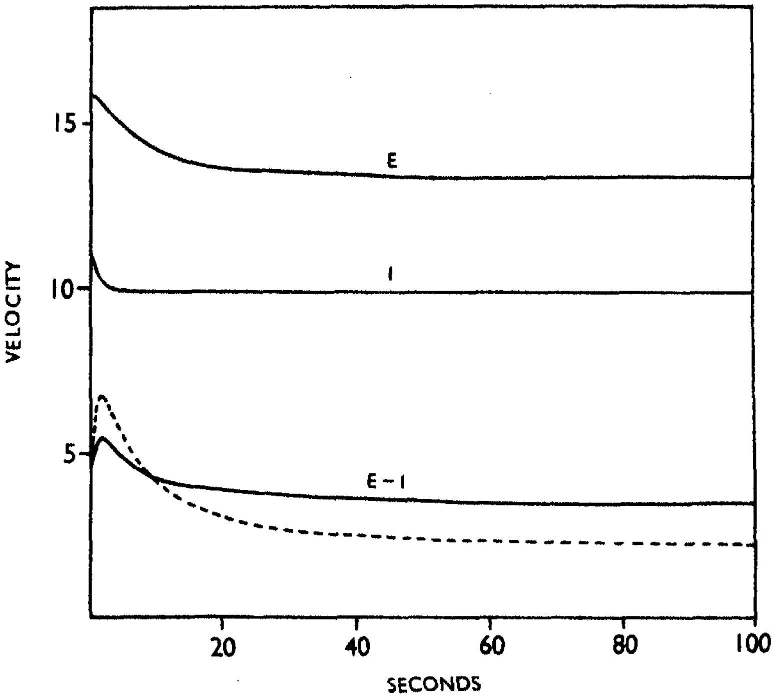

FIG. 23. Explanation in text.

represent the course in time of the two processes during the 100 sec. following a drop in temperature from 15 to 13°. They are calculated from the empirical formulae

$$E = E_{15} + \frac{E_{13} - E_{15}}{R^{1/t}} \quad \text{and} \quad I = I_{15} + \frac{I_{13} - I_{15}}{r^{1/t}},$$

where E_{13} and E_{15}, I_{13} and I_{15} are the equilibrated velocities of *E* and *I* at 13 and 15° respectively, t = time after the temperature drop, and *R* and *r* have the significance of time-factors which govern the rate of kinetic equilibration of the reactions. The values $R = 150$ and $r = 1·5$ have been arbitrarily taken. The curve for $E-I$ then has the form shown, and resembles the time course of the sensory discharge following a drop in temperature. The dashed curve is plotted for the values of $E-I$ following a greater temperature drop from 15 to 10°. As with the sensory discharge, the initial "response" is greater and the final equilibrated value is lower than with the smaller temperature difference. Following a rise in temperature the curves would be mirror images of those here shown, and the shape of $E-I$ would bear a similar resemblance to the sensory effect.

The processes E and I may be conceived as chemical reactions resulting in the production of excitatory and inhibitory substances, or they may represent more physiological quantities like excitatory state and threshold, and no doubt other possibilities could be imagined. But whatever the underlying mechanism of the rhythmical discharge may turn out to be, I think it can be maintained with some confidence that if the observed phenomena are to be interpreted in terms of simple underlying processes whose course in time after a temperature change is monotonic, such an interpretation will involve the conception of a *time factor of kinetic equilibration*, whose magnitude will be different for the different processes composing the system. The only instance of the occurrence of such a time factor that is known to me is in connexion with the viscosity of gelatin solutions, which, as Loeb (1922) showed, continues to increase at certain temperatures for many minutes after *thermal* equilibrium has been established. Possibly the frequency of the sensory discharge from the ampullae of Lorenzini is connected with the viscosity of some component in the system.

Biological significance of the results. The qualitative similarity in the behaviour of the ampullae, lateral-line organs and stretch receptors in relation to temperature changes may be taken as further evidence of the relationship of the biophysical structure of their excitatory systems. They are already related by their ability to maintain persistent rhythms of discharge, rhythms which in the intact animal apparently never cease throughout life, except when subjected to an inhibitory stimulus. (Stretch receptors in rays are always found to be rhythmically active when the muscles *in situ* are in the normal position of rest.) They are further related by the fact that their persistent rhythm is subject to inhibition or excitation by antagonistic phases of the appropriate physical disturbance (see Sand 1937; Fessard and Sand 1937). It is possible that in the mechanism of their reaction to temperature they may be found to be related to rhythmical and tonic muscles, for Mangold (1926) has described a paradoxical slowing of the isolated Carcinus heart in response to warming, and

Christ (1929), working with isolated strips of bovine carotids, showed that a sudden cooling through 10° resulted in a contraction followed by relaxation, while the contraction induced by a rise in temperature was sometimes preceded by a brief relaxation. Whether these phenomena bear any relation to the behaviour of sensory rhythms cannot at present be decided.

Whatever meaning is ultimately assigned to the qualitative similarity in the behaviour of the three sensory rhythms, there can be little doubt as to the significance of their *quantitative* differences. The magnitude of the responses of lateral-line receptors and stretch receptors to temperature changes is minute compared with the responses of the ampullae of Lorenzini. Fig. 24 may serve for the comparison of the maximal responses of which the three sensory systems are capable. The three curves are reproduced to one scale from preceding figures. Two degrees is very nearly a maximal stimulus for ampullae of Lorenzini. The best preparations have shown marked responses to one or two tenths of a degree, temperature differences which I myself could scarcely appreciate when I placed my finger in the position of the preparation, in contact with the surface of the glass bulb. Such sensitivity unquestionably raises the temperature responses of the ampullary end-organs to the rank of a specific sensory function. Hoagland (1935) demonstrated the acceleration of the discharge from lateral-line receptors with increasing temperatures in a manner similar to my experiments of fig. 20, and drew from this the conclusion that "It would seem, therefore, that one function of the lateral-line system is that of thermal reception" (p. 70). It cannot be too strongly emphasized that such variation of a sensory rhythm in function of temperature proves nothing more than that sensory rhythms, like all kinds of other rhythmical biological processes, must possess a positive temperature coefficient. Hoagland's reasoning would apply equally well to all three receptors that I have considered, and would lead to the absurd conclusion that ampullae of Lorenzini, lateral-line organs and stretch receptors are all concerned with the function of thermal reception. In the ampullae of Lorenzini we are confronted with a system whose variations in relation to *steady* temperatures are insignificant in comparison with its reactions to temperature *changes*. Such changes produce enormous and almost instantaneous responses which have all the characteristics of specific sensory action. It is a system which, like most sensory systems, is admirably designed to emphasize the effects of change. It is common experience that, by virtue of their adaptation, our own temperature receptors are poor thermometers; they are specialized for the detection of relative changes in temperature. The activity of the ampullae of Lorenzini is of the same kind.

In structure and disposition the ampullae of Lorenzini do not appear, at first sight, to be suitable structures to serve as temperature receptors. The end-organs are placed deep below the skin, and the long ampullary canals, filled with jelly, opening to the exterior by surface pores, appear to play no part in the temperature responses. The ampullary end-organs respond to temperature changes just as well whether the canals are *in situ* or not. Thus it is not possible at present to assign any functional significance to the canals. On the other hand the relation of the ampullary groups to the

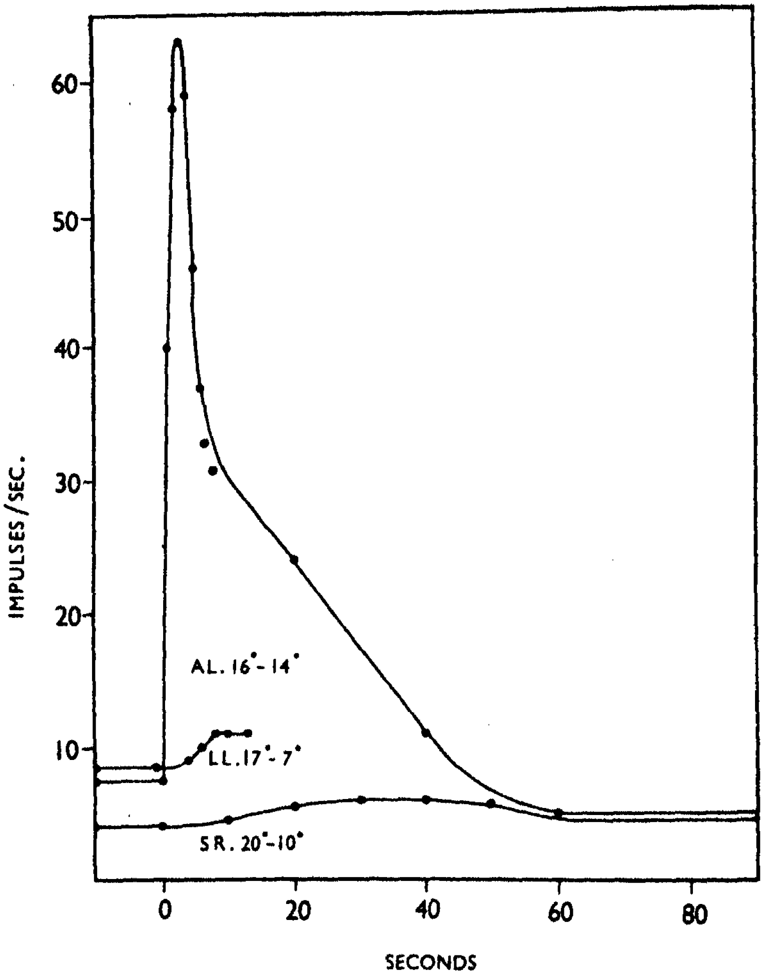

FIG. 24. Maximal responses of ampullae of Lorenzini (*AL*), lateral-line receptor (*LL*) and stretch receptor (*SR*) to cooling.

respiratory water current is striking. In the ray, the superficial ophthalmic, inner and outer buccal ampullae lie in the thin flat rostrum, the mandibular group (my preparation) is just under the skin below the corner of the mouth, and the large hyomandibular group lies close up against the anterior wall of the branchial chamber. In respiration these surfaces are bathed by the stream sucked in through the mouth and spiracle and expelled through the gills, and partly through the mouth. I have satisfied myself that the ophthalmic, buccal and mandibular ampullae can respond *in situ* to small temperature changes at the surface of the skin, the only difference being that there is possibly a slight delay in their response. There do not appear to be any cutaneous thermo-sensory endings in rays. I have explored skin-nerve preparations from various parts of the body, and have failed to find any evidence of receptors sensitive to temperature, although these preparations contained numerous tactile endings. It must be concluded, therefore, that the ampullae of Lorenzini are the only temperature receptors that these animals possess.

It is unlikely that the temperature receptors of higher vertebrates, even of teleostean fishes, will be found to be related in any way to the ampullae of Lorenzini of elasmobranchs and holocephali. Typical Lorenzini ampullae *have* been described, by Friedrich-Freska (1930), in one teleost, the tropical marine siluroid, *Plotosus anguillaris*.* But their presence in teleostean fishes is certainly very rare, and it seems probable that in the evolution of the land vertebrates the ampullae of Lorenzini have entirely disappeared.

Part of the apparatus was provided by a grant from the Government Grant Committee of the Royal Society.

SUMMARY

The histological structure of the ampullae of Lorenzini is still in dispute, though there is strong evidence that they are not neuromasts. The central connexions of their innervation have not been thoroughly studied. Their functional activity, as revealed by the present oscillographic investigation, is entirely distinct from that of lateral-line organs. For these reasons their unqualified inclusion in the acustico-lateralis system is unwarranted.

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* Their occurrence in this form is interesting because Plate (1924) has supported his view that the ampullae of Lorenzini are pressure receptors by pointing out that they occur only in groups which have no swim-bladders. *Plotosus*, however, like all siluroids, has a swim-bladder, in addition to its ampullae of Lorenzini.

Like acoustico-lateral receptors they possess an autonomous rhythm of activity, but in contrast with the former, they are unaffected by any form of mechanical stimulation. Their sensory sensitivity is confined to thermal stimulation.

Their responses to temperature changes are highly peculiar. They respond to cooling by an acceleration, to warming by an inhibition of the rhythm of impulse discharge. Such responses have been shown to occur throughout the physiological temperature range, from 3 to 20° approximately, though it is probable that they are most sensitive in the region 10–15°. In this region clear responses have been recorded from isolated preparations to temperature differences of one or two tenths of a degree.

After a response to a temperature change, adaptation occurs, and a regular slow rhythm of discharge is re-established. Such adapted frequencies plotted against temperature yield the familiar type of temperature curve with a positive temperature coefficient.

Lateral-line and stretch receptors of *Raja* show a similar paradoxical behaviour with respect to temperature changes, but on an entirely different scale. Stimuli of the order of 10° are required to produce an effect, and their maximal responses, compared with those of the ampullae, are minute. This is interpreted as meaning that the paradoxical effect of temperature changes is common to sensory rhythms in general, and possibly to some tonic muscles as well, but that in the ampullae of Lorenzini the mechanism has become specialized and sensitized for the performance of a specific thermo-sensory function.

The general form of a hypothesis to account for the paradoxical temperature effect is suggested. It involves the assumption of underlying reactions with different time factors of kinetic equilibration.

No cutaneous thermo-sensory endings have been found in rays, and it is concluded that the ampullae of Lorenzini are the only temperature receptors of elasmobranch fishes.

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DESCRIPTION OF PLATES 33–36.

(See p. 542)

FIG. 1. A few of the hyomandibular ampullae of *Raja*. Scale 5 mm.

FIG. 2. Isolated preparation of the mandibular capsule. The nerve has been reduced to a few fibres. Ampullary canals cut across. Lateral-line branch can be seen by-passing the capsule. Scale 5 mm.

FIGS. 4, 5, 6, 7, 13, 16, 17 and 18. In all oscillographic records the time signal is in seconds. The white line below the base line marks changes in temperature. Its upward displacement signals a flow of cold water, its downward displacement a flow of warm water through the temperature chamber. Artefacts marked ×. Further explanations in text.

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On the blood circulation and metabolism of sabellids

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Sabellid worms are distinguished by having the respiratory pigment chlorocruorin in their blood. A series of communications has dealt with the properties of chlorocruorin and with peculiarities of the blood system of worms possessing this pigment (Fox 1926, 1932, 1933, 1934; Fox and Roche 1933; Roche and Fox 1933). In the present paper various aspects of the blood circulation, the functions of the tube and the metabolism of sabellids will be considered. The work was done at the Station maritime de Biologie, Tamaris-sur-Mer, on the Mediterranean coast of France, and at Birmingham with animals sent from Plymouth. I wish to thank Professor H. Cardot, Director of the Tamaris Station, most heartily for his welcome and help. The experiments at Tamaris were done with *Spirographis spallanzanii* Viviani and those at Birmingham with *Sabella pavonina* Savigny and *Dasychone bombyx* (Dalyell). Young *Spirographis spallanzanii* were also used in Birmingham for the experiments on tropisms.

1. Blood circulation and metabolism

The blood circulation of sabellid worms has already been briefly described (Fox 1933). A fuller description will be published later by Mr D. W. Ewer of this Department. There is a peri-intestinal sinus leading anteriorly into a short dorsal vessel, which is connected by circum-oesophageal vessels with a ventral vessel. From the circum-oesophageal vessels a trunk goes forwards into each side of the crown, subdividing into a branch running along each pinna, with a blind-ending side branch in every pinnule. In each abdominal body segment, as seen from fig. 1, the ventral vessel is joined to the peri-intestinal sinus by a pair of ring vessels, from each of which a branch, the lateral connective, leads into the lateral vessel which runs dorso-laterally along each side of the worm. On either side of each segment of the abdomen the lateral vessel gives off a blind-ending dorsal branch, and a branch, leading towards the notopodium, which ends in blind-ending capillaries close to the surface of the body. These capillaries, above and in front of the notopodium, are referred to below as the notopodial capillaries. There are numerous blind-ending capillaries projecting into the coelom and others supplying the glandular parts of the body wall, but the muscles are devoid of capillaries. It is surprising that the glands should have a blood supply while the muscles have none.

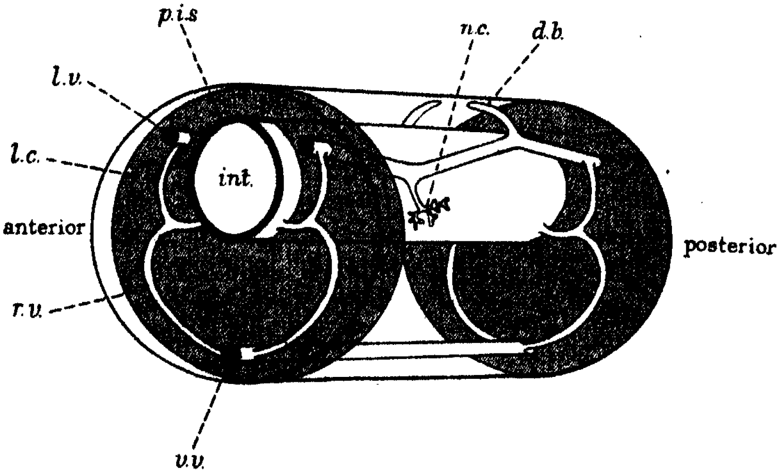

Fig. 1. Digram of the abdominal blood vessels of *Sabella*. The body wall is represented as if transparent, and the septa, which are stippled, are shown as if semi-opaque. The back of one segment is shown, and the whole of the segment posterior to it. The cut ends of blood vessels are represented in black. *d.b.* dorsal branch of lateral vessel; *int.* intestine; *l.c.* lateral connective; *l.v.* lateral vessel; *n.c.* notopodial capillaries; *p.i.s.* peri-intestinal sinus; *r.v.* ring vessel; *v.v.* ventral vessel.

Almost all the blood vessels, both trunks and blind-ending capillaries, are rhythmically contractile. In a small translucent sabellid the contractions of peri-intestinal sinus, lateral vessels, crown vessels, notopodial capillaries, and capillaries in the ventral gland shields, can easily be observed, and the movements of the ventral vessel and ring vessels can also be seen. The contractions of the coelomic capillaries can only be watched by opening the body wall of a large worm (Fox 1932, p. 483).

Blood is propelled forwards in the peri-intestinal sinus, from which it flows into the short dorsal vessel, and thence through the circum-oesophageal vessels into the ventral vessel, in which it is propelled backwards. Blood is propelled forwards in the two lateral vessels. Each lateral vessel is not full of blood all the time; blood in a section of the vessel is forced forwards, the walls of the vessel closing for some distance behind that section which is full of blood. Then another lot of blood enters the vessel from behind. There is thus a rhythmic progression forwards of successive trains of blood. The period is the same in the two lateral vessels, but their contractions are not synchronous on the two sides of the body (cf. the two halves of the crown). The notopodial capillaries fill themselves as each train of blood in the lateral vessel comes opposite them and they empty again when the train of blood passes on. There is no such regularity about the filling and emptying of the capillaries in the ventral gland shields (Fox 1933, p. 482).

In the abdomen blood flows from the ventral vessel into the pair of ring vessels of each segment.* The peri-intestinal sinus contains more and more blood from behind forwards; this blood has come from the ventral vessel through the ring vessels. In small worms the lateral vessels can be seen to fill up with their trains of blood at the posterior end of the worm; this blood has presumably come from the ventral vessel, through the lower part of the ring vessels and then through the lateral connective vessels.

That the blood of sabellids really circulates round the body, and is not merely moved to and fro by the peristalsis of the trunks and the rhythmic closing and opening of the blind-ending capillaries, is established by several observations. As just stated, it can be seen that blood is actually moved forwards in the lateral vessels, and that the notopodial capillaries connected to these vessels expel their blood as each train of blood moves forwards in the lateral vessel. When the notopodial capillaries open again it is fresh blood from the next train which fills them. There is evidence too that the blood which is expelled from the crown of a sabellid by each contraction of the crown vessels actually flows, in part at least, into the body, and does not merely accumulate in the short wide vessel at the base of each side of the crown, to surge back into the crown vessels when these relax again, for it can be seen in a small sabellid that the blood empties from the crown into the circum-oesophageal vessels through which blood is being propelled to the ventral vessel. Moreover, the crown is a respiratory organ, for its amputation reduces the oxygen consumption of the worm very considerably (see below). Consequently oxygen must be transported from the crown to the body by circulating blood.

The rates of rhythmic contraction of the various blood vessels are different from one another. The fact that the rate of contraction is much more rapid in the peri-intestinal sinus than in the crown vessels has already been pointed out (Fox 1933, p. 481). Since then, average periods, in seconds, of contractions in *Sabella* at 19° C. have been found to be as follows: crown vessels 10·1, lateral vessels 8·4, ventral vessel 5·5, peri-intestinal sinus 2·5. The ventral vessel is the only one of these vessels in which the periods are irregular. Average periods, in seconds, for *Dasychone* at 18° are: crown vessels 11·7, lateral vessel 9·5, ventral vessel 5·9, peri-intestinal sinus 2·3. The order of the rates of contraction is thus the same in the two forms.

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* The statement in Fox (1933, p. 480) that the blood flows "outwards in the segmental ring vessels which connect the sinus with the ventral vessel" is incorrect.

Within a given species the periods of contraction of the blood vessels are slower in large than in small individuals. In *Spirographis*, for instance, mean periods of contraction of crown vessels at 22° were as follows: 14·3 sec. for seven large worms having a mean individual wet weight of 12·6 g., and 10·7 sec. for nine small worms having a mean individual wet weight of 0·31 g. This is comparable with the fact that the rate of heart beat of prawns is quicker in small than in large individuals (Fox and Wingfield 1937, p. 281).

As mentioned above, although the large glands in the body wall of these sabellids are provided with capillary blood vessels, there are no capillaries in the muscles. The muscles must therefore derive their oxygen from that dissolved in the coelomic fluid and in the sea water in the worm's tube. This apparently inefficient mode of oxygen supply to muscles may account for two peculiarities of sabellids, namely, the large number of blind-ending capillaries in the coelom, and the high chlorocruorin content of the blood. It is remarkable that such relatively sluggish animals as sabellids have a higher total oxygen capacity of the blood than that hitherto found in any other invertebrate (Fox 1926). This high oxygen capacity of the blood, and the rich blood supply to the coelomic fluid through the numerous capillaries, would tend to keep the coelomic fluid saturated with oxygen, and so supply the muscles as adequately as the anatomical arrangement allows. The relatively high unloading tension of the respiratory pigment (Fox 1932) would assist this oxygen supply to the muscles through the intermediary of the coelomic fluid. The coelomic capillaries, however, may also be required to supply oxygen to the genital products in the breeding season; and digested food must reach the muscles from them.

One of the most remarkable things about the blood circulation of sabellid worms is that after a worm has retired into its tube for a short time, the contractions of the vessels in the crown cease (Fox 1933, p. 483). I have now studied the stoppage of contractions of blood vessels in the body of the worm when it is in a tube. This was done by putting small translucent individuals of *Sabella* into glass tubes. It is not necessary to cork the tubes, for, provided that the worm remains completely within the tube and not even the tip of the crown projects beyond the end, the contractions of the vessels eventually stop. As explained above, the peri-intestinal sinus and the lateral vessels with their accompanying notopodial capillaries can easily be observed through the body wall. The experiments with the worms in glass tubes showed that the peri-intestinal sinus stops contracting just before the vessels in the crown stop, or at the same time as they do, but the lateral vessels with their notopodial capillaries do not stop until later. To explain the stoppage of the crown vessels when the worm is in its tube I previously put forward the hypothesis that carbonic acid accumulates between the closed crown filaments and causes the contractions to cease (Fox 1933, p. 488). The data just related for the times of stoppage of the vessels in the body are not in disagreement with this hypothesis: carbonic acid would accumulate more rapidly in the peri-intestinal sinus, which is far from the surface on the worm, than in the lateral vessels with their superficial notopodial capillaries. It is true that the contractions in the crown vessels stop almost as soon as those in the peri-intestinal sinus, although the crown vessels are near the surface, but the crown has a high rate of metabolism (see below) and would therefore produce a lot of carbon dioxide.

After a sabellid has retired into its tube and its blood circulation has stopped, much less oxygen will be available to the tissues. It is of interest therefore to know whether the oxygen is rapidly unloaded from the oxychlorocruorin. If a *Sabella* in a glass tube at 18° is examined with a microspectroscope, the oxychlorocruorin α-band, although it becomes feebler, does not disappear either in crown or body up to 100 min. after the worm has been in the tube, although the contractions of the crown vessels and peri-intestinal sinus stop within less than half an hour. Presumably the slowness of the diffusion of oxygen out of the stationary blood prevents the rapid reduction of the oxychlorocruorin.

At the Marine Biological Station, Lough Ine, Co. Cork, Miss R. F. Griffith of this Department observed in August that in the sea most *Sabella* are retracted into their tubes in the daytime, whereas most of them have their crowns extended at night. McIntosh (1922, p. 230) had already noted that *Sabella* "is most active at night". I made quantitative observations on *Spirographis* at Tamaris in September. I noted the state of ten particular worms, living at a depth of 1 m. of water below a landing stage, at 5.45, 6.45, 7.45 and 9.45 a.m. on 14 days. On the first day the sun rose at 5.5 a.m., on the last day at 5.28 a.m. The sky was always cloudless and the water clear. The percentages of worms with crowns extended at the four times stated were 81, 51, 34 and 23 respectively. It is clear that this is a series tending towards 100% with crowns extended at night. These sabellids, then, feed mostly at night, and in the daytime the blood circulation of most of the worms must be stopped while they are retracted in their tubes, unless they periodically protrude the tip of the crown from the tube.

2. INFLUENCE OF THE TUBE ON VIABILITY AND METABOLISM*

Spirographis and *Sabella* live in tubes made of a mucoid secretion mixed with mud (Nicol 1930). Removed from their tubes they are incapable of making new ones. But Dalyell (1853) discovered that if just the hind end of the tube is cut off, the animal protrudes its tail and makes a new hind end attached to the ground. He also observed that within 24 hours the animal raises its tube up from the prone position, and also curves the tube. Loeb (1890) found that *Spirographis* is negatively thigmotropic and positively phototropic. I have confirmed all these observations with small *Spirographis*. The animals attach the cut hind end of the tube to the ground, then curve the front end away from the ground, sometimes raise the whole tube up, and sometimes curve the tube towards the light. By attaching animals with rubber bands to microscope slides, and fixing the slides in different positions, I found that the curvature away from the ground is due both to negative thigmotropism and negative geotropism.

The tubes of *Spirographis* and *Sabella*, after the animals have been removed from them, cannot be stretched, but they can easily be bent. The tubes, however, are completely elastic in that they return at once to their initial shape after having been bent. In spite of this, the animals can permanently bend the tubes into new curves. How is this done? Loeb (1890) pointed out that the innermost layer of the tube is the most elastic, and he made this fact responsible for the curvatures. I consider that this is only part of the cause. When an animal raises its tube up from the ground, we have a lever system of the 3rd class; and the effort may well be supplied by secretion, on the side away from the ground, of elastic tube substance which would contract after having been secreted. Permanent curvatures of the tube into new shapes could not, however, be explained in this way. To be curved, the tube must be softened, and it seems to me probable that the power which the animal has of digesting its tube (Fox 1933, p. 484) may well bring this about. A combined action of softening by digestion, and secretion, on the concave side of the curvature, of elastic tube material which contracts, would explain the facts. I had previously concluded that the digestive secretion is produced by the anterior end of the worm, but tubes can be curved in any part of their length, so if the explanation just given is correct, the secretion cannot be produced at the front end alone.

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* A preliminary account of part of this section appeared in *Nature, Lond.*, **141**, 1937, p. 163.

Sabella and *Spirographis* possess two kinds of glands in the body wall of each segment: they have ventral glands (gland shields) and lateral glands. The ventral and lateral glands differ in that the ventral glands are richly supplied with blood capillaries while the lateral glands have few. If the ventral glands secrete the lining of the tube, the lateral glands may be responsible for its softening by digestion. This suggestion that the two kinds of glands are responsible respectively for secreting and softening the tube is supported by the nature of the glands of *Myxicola infundibulum* (Renier). This worm secretes gelatinous mucus only and it has glands of a single kind, which extend all round the body wall. Since *Myxicola* does not make a solid tube it has no need for other glands to furnish a softening enzyme.

Spirographis and *Sabella* can sometimes be persuaded to leave their tubes by pressure with the fingers on the posterior end of the worm. If this fails, the tube can be slit open by inserting a long metal probe, grooved on one side, between the worm and its tube; the groove is turned towards the tube and a scalpel is run along the groove. Removed from their tubes, *Spirographis* and *Sabella* are less viable than when intact. In an aquarium with an average temperature of 20°, thirteen tubeless *Spirographis* died in 10 days, whereas worms in their tubes were kept alive indefinitely in this aquarium. In an aquarium at 23°, twelve tubeless worms died in 7 days, and in another aquarium at 25·5°, eight tubeless worms died within 6 days, while worms with tubes remained healthy in both aquaria. The mode of death of naked worms is peculiar. Patches resembling sores appear, extending over several segments. At these places the body wall breaks down and the intestine becomes visible. Shortly afterwards the worms die.

Tubeless worms can be kept alive indefinitely in fully aerated water; they died in the aquaria because, by their own respiration, they reduced the oxygen content of the water. In an experiment at 23°, six tubeless *Spirographis* were put into an aquarium in which the water contained 4·8 c.c. oxygen/l. On the 3rd, 4th, 5th, 6th and 7th days the oxygen contents of the water were respectively 3·6, 3·7, 2·6, 2·4 and 2·8 c.c./l. (av. 3·0); on the 4th, 5th and 7th days, 1, 1 and 4 worms died. In another experiment at 23°, six tubeless worms were put into an aquarium with an oxygen content of 4·9 c.c./l. On the 3rd, 4th, 5th, 6th and 7th days the oxygen contents of the water were 4·0, 2·0, 1·6, 0·7 and 1·9 c.c./l. (av. 2·0); on the 4th, 5th, 6th and 7th days 1, 1, 3 and 1 worms died. In both these experiments worms with their tubes remained healthy in the same aquaria.

In water deficient in oxygen, which causes the death of tubeless *Spirographis* and *Sabella*, worms can be kept alive by putting them into glass tubes. The hind end of the glass tube is closed by a cork. The worms line the glass tubes with a reddish translucent layer like the internal lining of their own mud tubes, and they lengthen the front end of the glass tubes with normal tube material made with mucous secretion and mud. If the cork closing the hind end of the glass tube has a hole bored in it, the end of the worm projects through the hole from time to time and gradually attaches the glass tube to the floor of the aquarium with hardened mucous secretion like the hind end of a natural tube. It is only during the first 24 hours that the worms tend to leave the glass tubes; this can be prevented by closing the front end of the tube with a bored cork during this period. By the end of 24 hours the worm has commenced to line the tube, and usually no longer leaves it when the cork is removed from the front end.

By watching *Spirographis* in glass tubes it can be seen that the worm performs rhythmic body movements. At regular intervals a swelling of the body wall, apparently completely filling the tube, forms at the hind end of the worm. This swelling moves forwards. When the swelling has reached the front end of the worm, the hind part of the worm's body is drawn backwards and a new swelling forms at the hind end of the worm, to move forwards in its turn. Occasionally the swellings form at the front end of the animal and move backwards, this reversed direction of motion being maintained for some time. From time to time there are quiescent periods. At 18·5° the interval between one pulsation and the next in large *Spirographis* was found to be 16·1 ± 0·2 sec. (mean and standard error of 47 values).

Neither oxygen deficit nor carbon dioxide excess in the water influences the rhythmic body movements; at 18·5° the movements of *Spirographis* continued with the same frequency while the oxygen in the surrounding water was gradually reduced by the respiration of the worms from 5·1 to 1·2 c.c./l. over a period of 2½ hr. Nor are the rhythmic movements of the body affected by the expansion or retraction of the crown.

It is probable that the rhythmic body movements renew the water in the tube, although the hind end of the tube is closed, at least in *Sabella* which has the hind end of its tube buried in mud. Worms removed from their tubes only occasionally execute these rhythmic movements of the body wall, and when the movements occur they are feeble compared with the rhythmic movements of a worm in its tube. Tubeless worms, in water deficient in oxygen, may die of oxygen-lack because the water in immediate contact with the body is not renewed by body movements, although the water in contact with the crown, which accounts for two-thirds of the oxygen intake by the animal (see below), is presumably still renewed by ciliary action. But tubeless worms in oxygen-deficient water may perhaps die owing to the absence of body movements allowing semi-anaerobic bacteria or fungi to settle on the body. In fully aerated water the naked worms must receive sufficient oxygen, either for respiration or to avoid infection, in spite of the absence of vigorous rhythmic body movements; they are therefore able to live. Worms with their tubes doubtless require to execute the body movements even in aerated water owing to the small amount of water in the tube and the difficulty of renewing it through the front opening of the tube and so keeping it oxygenated. It is apparent then that the tube of *Sabella* and *Spirographis* has a second function in addition to that of protection: it seems to provide the mechanical stimulus for the reflex body movements necessary to the animal with its tube.

Since tubeless *Spirographis* do not execute the vigorous rhythmic body movements, it is of interest to know whether there is any considerable change in metabolism after the animals have been deprived of their tubes. To find this out the oxygen consumption of the worms was first determined. Each experiment was done with a single worm, at first with its tube and then removed from its tube. After algae and sessile animals had been scraped off the tube, the worm, in its tube, was put into a flat-bottomed wine bottle full of sea water, the hind end of the tube being towards the neck of the bottle. The bottle was corked and put bottom upwards in a water thermostat. Several short pieces of glass rod were in the bottle, which was turned over every quarter of an hour to agitate the water. The worms kept their crowns expanded in the bottles even after the water had been agitated.* The oxygen content of the water was determined before the experiment. At the end of the experiment, which lasted an hour, water was siphoned out of the bottle for another oxygen determination. Then the worm was removed from its tube and the experiment was repeated, determinations of the oxygen consumption of the tubeless worm being made on the same day and again on the next day. In the case of the tubeless worm, the wine bottle was laid on its side. The oxygen content of the water was determined by Nicloux's (1930) modification of the Winkler method.† The results of the experiments are given in Table I. The mean percentage decrease in the oxygen consumption of worms after removal from their tubes was 27 on the first day and 49 on the second day. In other words the oxygen consumption fell to three-quarters of its initial value on the day that the worms were removed from their tubes and to one-half on the next day.*

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* Such an experiment cannot be done with *Sabella*, which is more sensitive to mechanical stimuli and retires into its tube when disturbed.

† The method can be simplified without loss of accuracy by using a straight tube instead of the Y-tube (Fox, Wingfield and Simmonds 1937, p. 212).

TABLE I. OXYGEN CONSUMPTION OF SINGLE INDIVIDUALS OF *SPIROGRAPHIS SPALLANZANII* WITH AND WITHOUT THEIR TUBES. TEMP. 21°

Worm no.	State of worm and time	Oxygen consumption c.mm./c.c. worm/hr.	% decrease
1	With tube	102	—
	Tubeless, same afternoon	79	23
	Tubeless, next morning	54	47
2	With tube	81	—
	Tubeless, same afternoon	59	27
	Tubeless, next morning	38	53
3	With tube	107	—
	Tubeless, same afternoon	74	31
	Tubeless, next morning	56	48
		Mean: same afternoon,	27
		next morning,	49

The ammonia excretion of *Spirographis* was also measured, both in its tube with crown expanded, and out of its tube. For each experiment an animal was put in an upright glass cylinder immersed in a water thermostat. The crown remained expanded throughout the experiment, which lasted 1½–3½ hr. The ammonia content of the water was measured at the beginning and end of the experiment by the Nessler method. For Nesslerization the sea water was distilled after addition of sodium carbonate. Allowance was made for ammonia present in the sea water before the experiment. Immediately after the determination of the ammonia production of the worm in its tube with its crown expanded, the second part of the experiment followed, the animal being removed from its tube and its ammonia production again measured. The results are given in Table II. The mean percentage decrease in ammonia excretion after removal of the worms from their tubes is 20: the excretion falls to four-fifths of its former value. This is comparable with the fall in oxygen consumption under the same circumstances to three-quarters of its initial value.†

* There is no change in the rate of pulsation of the blood vessels in the crown either on the day that *Spirographis* is removed from its tube or on the following day.

† Animals were used which had been in the aquarium for several days. The fall in metabolism was therefore not due to a progressive harmful effect of the aquarium environment.

The decrease in the metabolism of worms deprived of their tubes is probably due largely to the absence of vigorous rhythmic body movements in these animals.

TABLE II. AMMONIA EXCRETION OF SINGLE INDIVIDUALS OF *SPIROGRAPHIS SPALLANZANII* WITH AND WITHOUT THEIR TUBES. TEMP. 22°

	Nitrogen, mg./c.c. worm/hr.		
Worm no.	With tube	Tubeless	% decrease
4	46	32	30
5	35	27	23
6	38	25	34
7	20	20	0
8	21	17	19
9	28	19	32
10	26	21	19
11	70	58	17
12	40	36	10
13	53	35	34
14	30	26	13
15	57	55	4
			Mean 19·6 ± 3·3*

* Standard error.

3. THE RESPIRATORY FUNCTION OF THE CROWN

The crown of *Sabella* and *Spirographis* is an organ for collecting food and for gathering mud to make the tube (Nicol 1930). It is also apparently a respiratory organ. The effect on the metabolism of the worm of amputating the crown was first tested experimentally by Bounhiol (1902) and then by Zoond (1931) who found that the oxygen consumption of tubeless *Bispira volutacornis* (Montagu) fell to 37 % of its former value after amputation of the crown. I have repeated the experiments of Zoond using tubeless *Spirographis* and have extended them to worms with their tubes. The technique was the same as that described in the last section. After the oxygen consumption of the animal with its crown had been determined, the crown was amputated and the oxygen consumption of the crownless worm was measured immediately afterwards. In the case of intact worms having both tube and crown, the crown remained expanded throughout the experiment. The results are given in Table III. The mean percentage decrease in oxygen consumption after amputation of the crown is 63 for animals with their tubes and 64 for those out of their tubes. The respiration thus falls to 36–37 % of its previous value. This agrees with Zoond's figure for tubeless *Bispira*. The oxygen intake by the body of the worm must be largely effected by the notopodial capillaries.

TABLE III. OXYGEN CONSUMPTION OF SINGLE INDIVIDUALS OF *SPIROGRAPHIS SPALLANZANII* WITH AND WITHOUT CROWNS. TEMP. 21°

		Oxygen consumption c.mm./c.c. worm/hr.		
Worm no.	State of worm	With crown	Crownless	% decrease
1	Tubeless	54	16	70
2	,,	38	17	55
3	,,	56	18	68
				Mean 64
16	With tube	110	19	83
17	,,	89	33	63
18	,,	72	30	58
19	,,	45	23	49
				Mean 63

It was next necessary to know whether the very considerable fall in oxygen consumption after amputation of the crown is due to a high rate of respiration of the crown itself or to the elimination of a respiratory organ. Does the crown, owing to its own metabolism, account for 63% of the oxygen consumption of the animal? To answer this question measurements were made of the oxygen consumption of isolated crowns of *Sabella* immediately after amputation. In this instance the determination of oxygen was made by a method for use with small quantities of water (Fox and Wingfield 1938). A single individual was used for each experiment and 21 experiments were made. The experimental temperature was 17° and the water was saturated with air at the beginning of the experiment. The oxygen consumptions of the amputated crowns (c.mm. oxygen/g. *wet* weight of crown/hr.) in the separate experiments were as follows: 212, 264, 231, 263, 209, 197, 276, 209, 166, 152, 78, 103, 83, 129, 116, 101, 146, 96, 126, 189, 156. The mean value is 167. The ratio of the wet weight to the dry weight of the crown of *Sabella* is 6·9 : 1 (based on seven determinations, the extreme values of which were 6·6 and 7·2). It follows that the mean oxygen consumption of the crown in c.mm./g. *dry* weight of crown/hr. is $167 \times 6{\cdot}9 = 1152$. This may now be compared with the oxygen consumption of whole worms. The mean oxygen consumption at 17° of whole individuals of *Sabella* just removed from their tubes, in waters containing 1·3, 2·9, 4·4 and 10·2 c.c. oxygen/l., is 237, 359, 430 and

498 c.mm./g. dry weight/hr. respectively.* By interpolation from these data the oxygen consumption of the animals in air-saturated water is 450. The rate of oxygen consumption of the crown, namely, 1152, is thus 2½ times that of the whole worm. This high metabolism may perhaps be due to the ciliary activity or to the mucous glands of the crown. The ratio of the wet weight to dry weight of whole worms is 58·3 ± 1·1 (mean and standard error of fifty-one values). Therefore the oxygen consumption of whole worms in c.mm./g. wet weight/hr. is $450/58 = 78$. The wet weight of the crown is 13·6 ± 0·7 % of the wet weight of the body (mean and standard error of weights of crown and body of thirty-seven worms); therefore the ratio of the wet weight of the crown to that of the whole worm is 14 : 114. The oxygen consumptions of crown and of whole worm are consequently in the ratio of $14 \times 167 : 114 \times 78$, or 23 : 89, and the ratio of the oxygen consumption of the body of the worm to that of the whole worm is $89 - 23 = 66 : 89$. Amputation of the crown should therefore reduce the oxygen consumption of the worm to $66 \times 100/89 = 74$ % of its original value. Actually (in *Spirographis*) it falls to 36 %. This decrease cannot therefore be accounted for by the high rate of metabolism of the crown.

The fall in rate of metabolism in sabellids after the crown has been amputated might be due to a disorganisation of the animal's bodily economy, as when a man is decapitated, or it might be caused by partial oxygen starvation of the worm's body after the removal of a respiratory organ. Since the principal ganglia are not contained in the crown, and since the animals autotomize their crowns, or lose them when fishes bite them off (Nicol 1930, p. 539), to regenerate them subsequently, the loss of the crown cannot be compared with the decapitation of a higher animal. It would appear, therefore, that the crown is an organ of respiration and that the loss of this function accounts for the lowered metabolism of the crownless worm. It is true that the crown is also a feeding organ, but the fall in metabolism occurs too soon after amputation of the crown to be due to starvation.

Sabellids lose their crowns in nature and regenerate them. When crownless, the oxygen consumption of the worms is only 36 % of its full value and it is remarkable, as pointed out by Zoond (1931), that animals should be able to exist at such different levels of metabolism. Sabellids, moreover, not only can live without the crown, until it is gradually regenerated, but when they are cut into pieces, each piece regenerates anteriorly and posteriorly and becomes a whole worm. I thought it of interest therefore to measure the oxygen consumption of pieces at intervals during the course of regeneration. The results of such an experiment are given in Table IV. The oxygen consumption of these regenerating pieces, as shown in the Table, may be compared with that of intact tubeless worms. The oxygen consumption at 15° of three individual intact *Sabella*, removed from their tubes 5 hr. previously, was 54, 52, 45 c.mm./c.c. worm/hr. The table shows that the respiration of the regenerating pieces starts at a low value and, as the new crowns increase in size, slowly rises to the value for intact worms lately removed from their tubes, and therefore above the value for worms removed from their tubes on the previous day. The relatively high metabolic rate attained by the regenerated pieces may be due to the fact that they execute much more active body movements than tubeless whole worms.

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* The details of the experiments from which these data are derived will be published later.

TABLE IV. OXYGEN CONSUMPTION OF NUMEROUS REGENERATING FRAGMENTS OF *SABELLA PAVONINA*. TEMP. 15°

Days after worms were cut up	16	19	20	21	22	26	28	30	34	41	43	48	51
Oxygen consumption, c.mm./c.c. worm/hr.	19	24	26	29	30	29	34	33	43	46	46	48	49

SUMMARY

Most of the blood vessels of sabellid worms are rhythmically contractile. The rates of contraction increase in the following order: crown vessels, lateral vessels, ventral vessel, peri-intestinal sinus. The rate of contraction is more rapid in small than in large individuals.

There is evidence that the movement of the blood is a circulation.

When *Sabella* retires into its tube the contractions of the peri-intestinal sinus stop just before those of the crown vessels, while the lateral vessels do not cease contracting until later. The oxychlorocruorin in the stagnant blood is not completely reduced.

There are capillary blood vessels supplying body-wall glands but none in the muscles. The muscles must therefore obtain oxygen from the sea water and from the coelomic fluid, which is richly supplied with blood capillaries. The high total oxygen capacity of the blood and the high unloading tension of chlorocruorin may be correlated with this indirect oxygen supply to the muscles from the blood through the coelomic fluid.

Spirographis is negatively thigmotropic, negatively geotropic and positively phototropic. These tropisms involve permanent curvatures of the tube which are probably accomplished by softening the tube through digestion, together with secretion of elastic tube substance.

Spirographis deprived of its tube will live indefinitely in aerated water, but dies in water deficient in oxygen, although worms with their tubes remain healthy in this water.

Spirographis deprived of its tube and put into a glass tube lines the glass tube with a secretion and lengthens it like a normal tube. Worms with these glass tubes remain healthy in water deficient in oxygen.

It can be seen from *Spirographis* with glass tubes that the worms with their tubes execute vigorous rhythmic body movements. These movements are unaffected by oxygen deficiency or carbon dioxide excess in the water.

The rhythmic body movements probably continually renew the water in the tube. These movements are feeble or absent in tubeless worms. It is probably for this reason that tubeless worms die in water deficient in oxygen, while worms with their tubes can live in water deficient in oxygen by continually renewing the water in contact with the body. Tubeless worms are viable in aerated water probably because they can obtain enough oxygen without body movements. These movements must be necessary to worms with their tubes, even when the surrounding water is aerated, because of the small lumen of the tube and the difficulty of renewing water through the anterior opening of the tube alone.

The tube has thus a second function in addition to being protective: it provides the mechanical stimulus for reflex rhythmic body movements necessary to the animal with its tube.

When *Spirographis* is removed from its tube, its oxygen consumption falls to three-quarters of the initial value on the same day, and to one-half on the following day, while its ammonia excretion falls on the day of removal from the tube to four-fifths of the initial value. This fall in metabolism must be due in part to the absence of vigorous rhythmic body movements in the tubeless worms.

When the crown of *Spirographis* is amputated, the rate of oxygen consumption of the worm falls to one-third of the value for the intact worm. This is true both of worms with their tubes and of those deprived of tubes.

The rate of oxygen consumption of the isolated crown of *Sabella* is 2½ times that of the whole worm, but this high rate of metabolism of the crown is not sufficient to account for the fall in oxygen consumption of the worm after the crown has been amputated. The crown is therefore a respiratory organ.

During the regeneration of fragments of *Sabella* the oxygen consumption slowly increases from a low initial value to that of the intact tubeless worm.

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END OF THE ONE HUNDRED AND TWENTY-FIFTH VOLUME (SERIES B)

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

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21 JANUARY 1938

Chemical structure in relation to oestrogenic activity. Compounds without the phenanthrene nucleus. By E. C. DODDS and W. LAWSON.

(*Communicated by Sir Henry Dale, F.R.S.—Received* 11 *December* 1937.)

The oestrogenic activity of calciferol and clupanodonic acid appeared to indicate that the phenanthrene nucleus was not necessary for oestrogenic activity. This view was substantiated by demonstrating the potency of various derivatives of acenaphthene.

A large number of other substances, such as carbinols, derivatives of diphenyl methane, diphenyl ethane and diphenyl ethylene have been shown to be oestrogenic. Compounds as simple as *p*-hydroxy propyl benzene and *p*-hydroxy propenyl benzene were found to possess full oestrogenic activity. The most potent substances, however, were found among the diphenyl ethylene derivatives.

The possibility of the formation of polymerides from *p*-hydroxy propenyl benzene was considered.

Quantitative spectrographic analysis of biological material. III.

By J. S. Foster, F.R.S., G. O. Langstroth and D. R. McRae. (*Received 14 December* 1937.)

An internal standard method of quantitative spectrographic analysis, as applied in the determination of Na and K in glandular secretions, is described. The probable error in a determination is about 4%.

By adding an appropriate foreign substance to the sample in such quantities that the character of the condensed a.c. spark discharge is determined by this substance, it is possible to use the same working curve for samples of considerably different composition.

The sample and internal standard may be separately placed on the electrode by a pipette specially designed for accurate delivery of small volumes. The volume of a sample required for the determination of both elements is thus reduced to 0·01 c.c.

By photographing the spectrum of the sample with an antimony absorption step-weakener before the spectrograph slit it is possible to obtain determinations for two or more elements from a single exposure.

The secretion of protein material in the parasympathetic submaxillary saliva.

By G. O. Langstroth, D. R. McRae and G. W. Stavraky. (*Communicated by J. S. Foster, F.R.S.—Received* 14 *December* 1937.)

A quantitative study has been made of the variations in composition which occur in the submaxillary saliva of the cat when the intensity of chorda tympani stimulation is altered. The relative concentrations of protein material were determined from the absorption spectrum of the saliva, while Na and K concentrations were determined from the emission spectrum. Less extensive analyses for other constituents were made chemically.

A mathematical theory of the secretion of protein material has been developed. It presents the following picture of the secretory processes. (1) During stimulation some activating substance is liberated within the gland; (2) this substance sets in operation the water secretion mechanism, controls the membrane permeability, and sets up a reaction, or chain of reactions, resulting in the transformation of granule material to a form readily carried out of the gland by the flow of water. It leads to the following expression for the relative concentrations of protein material in two samples (n, i) taken from a series in which the intensity of the stimulus is varied:

$$C_i/C_n = D_i/D_n . e^{-\sum_{n}^{i-1} kBr}.$$

k is a constant, D_i represents the concentration of (Na+K) in the ith sample in milliequivalents per litre, and B_r represents the amount of (Na+K) secreted in the rth sample in milliequivalents. The expression represents the observed results remarkably well. The theory is also in accord with certain other observed features of the secretory processes.

The theory of pressure-ionization and its applications. By D. S. KOTHARI. (*Communicated by M. N. Saha, F.R.S.—Received* 14 *December* 1937.)

The main results which have been obtained from the application of the theory of pressure ionization may be summarized as follows:

(i) The theory predicts that the stellar material in the interior of the white dwarf stars should be fully ionized.

(ii) It predicts that there cannot be a "cold" body (planet of white dwarf) larger in size than Jupiter.

(iii) The theory shows that the two heaviest planets (Jupiter and Saturn) have cores composed of metallic hydrogen. The terrestrial planets have cores of much heavier metal possibly iron.

Comparison of wave-functions for HeH^{++} and HeH^{+}. By C. A. COULSON and W. E. DUNCANSON. (*Communicated by E. N. da C. Andrade, F.R.S. —Received* 14 *December* 1937.)

As many different approximations as possible have been used in a comparative study of the wave-functions of HeH^{++} and HeH^{+}. In the latter ion, the molecular-orbital approximation is found to be much better than the electron-pair approximation. Ionic terms and polar terms are included in the final wave-function, and an expansion in terms of spheroidal co-ordinates is given. The lowest value obtained for the energy of HeH^{+} is $-2{\cdot}935$ A, with an internuclear distance of $1{\cdot}446$ A $= 0{\cdot}764$ A°. The dissociation energy certainly lies within 0·61 and 2·10 V, being probably about 1·5 V. The fundamental vibration frequency is 3380 cm^{-1}.

The constitution of heavy water. By I. RAMAKRISHNA RAO and P. KOTESWARAM. (*Communicated by O. W. Richardson, F.R.S.—Received* 14 *December* 1937.)

The Raman band of heavy water is found to be similar to that of ordinary water both in diffuseness and extent. The variations with temperature in the structure of this band are also similar to those of ordinary water. These changes are attributed to changes in the relative proportions of (D_2O), $(D_2O)_2$, and $(D_2O)_3$ molecules which are assumed to be in thermal equilibrium. By an analysis of the intensity curves of the band, the proportions of the three polymers at different temperatures are calculated. The results are compared with those of water and the points of similarity and differences explained on the basis of their other physical properties.

The production of γ-rays by neutrons.

By E. H. S. Burhop, R. D. Hill and A. A. Townsend. (*Communicated by T. H. Laby, F.R.S.—Received* 14 *December* 1937.)

Measurements have been made of the absorption in boron of those neutrons which excite γ-rays in specimens of cadmium, silver (of two thicknesses), arsenic, antimony, iodine and mercury. The measurements were carried out using thermal neutrons in the case of cadmium and silver (thin specimen) and non-thermal neutrons in the case of silver, arsenic, antimony and mercury.

The curves in the latter case were always of the same general type consisting of an initial component rapidly decreasing with boron absorber thickness superimposed on a component varying very slowly with boron thickness. It is shown that the initial component is to be associated with a nuclear resonance level corresponding to very small neutron energy, while the other component is due to neutrons captured into resonance levels of greater energy. The curves are interpreted in terms of the Bethe-Placzek formulation of Bohr's theory of nuclei.

In the case of silver, absorption measurements in boron have in addition been made of those neutrons which excite the γ-radiations of 22 and 138 sec. half-period.

The question of whether a nucleus which on neutron capture can emit both and γ-radiation will have the same resonance levels corresponding to both methods of reorganization is discussed. For silver the evidence points to the existence of separate sets of energy levels for γ-ray emission and γ-decay but it is not sufficiently conclusive to settle finally this point.

The development in vitro of the mammalian gonad. Ovary and ovogenesis.

By P. N. Martinovitch. (*Communicated by F. H. A. Marshall, F.R.S.—Received* 15 *December* 1937.)

Ovaries of young and embryonic rats and mice were successfully cultivated *in vitro* and a progressive differentiation of the germ cells obtained. The tissue was grown by the watch-glass method, the medium consisting of equal parts of fowl plasma and fowl embryo extract. In each case, one gonad was fixed as a control, whilst the other was cultivated *in vitro*.

If the ovaries are explanted at the 15th–16th day of embryonic development when primitive germ cells are only present and ovogenesis has not yet begun, rapid division of the oogonia followed by the typical synaptene stages of meiosis proceed uninterruptedly in the normal way. The diplotene phase of meiosis is succeeded by a resting stage when the individuality of the chromosomes becomes indistinguishable. Most of the resting oocytes remain unchanged until the end of the culture period (about 1 month), but some enlarge and after about 18 days' cultivation attain full size. Thus a primitive germ cell may develop *in vitro* from an oogonium into a full-size ovum. In the explants growth of the ovum is not always accompanied by growth of the follicle and an ovum may reach its full size in the complete absence of a follicle. New germ cells are not formed *in vitro* except by the mitotic division of the original oogonia.

The ovaries of older embryos and post-embryonic animals also develop readily *in vitro*.

The influence of wall oscillations, wall rotation, and entry eddies on the breakdown of laminar flow in an annular pipe.

By A. FAGE. (*Communicated by G. I. Taylor, F.R.S.—Received* 15 *December* 1937.)

(1) *Scope of work.* Experiments have been made to determine the effects of disturbances of known character on the laminar flow of water in a long pipe of annular cross-section. The disturbances considered are those due to axial oscillations of the inner wall of the pipe, to oscillations of the inner wall about its axis, and to both weak and intense entry eddies. Experiments on the breakdown due to a uniform rotation of the inner wall (outer wall fixed) have also been made.

The breakdown of flow near a plane surface oscillating in a stationary fluid has been observed.

Theoretical relations for the flow of a viscous fluid through an annular pipe, under the influence of a pressure gradient parallel to the axis, with the inner wall oscillating axially and the outer wall fixed have been obtained.

(2) *Conclusions.* The frequency of the axial oscillation of the inner wall when a departure from laminar flow occurs depends on the axial amplitude of the wall and the viscosity of the fluid, and is independent, within the accuracy of measurement, of the velocity of axial flow. The Reynolds number of disturbance, defined as the product of the velocity amplitude at the wall and a length $2\pi\sqrt{\nu/\pi f}$ (where f = frequency) divided by the viscosity, at which a departure from laminar flow occurs does not change appreciably over a wide range of amplitude.

The results with the inner wall of the pipe oscillating about its axis suggest that the flow remains laminar up to the critical Reynolds number of disturbance measured with the inner wall oscillating axially.

Visual observation suggests the presence of rotating bands of fluid with their axes parallel to the direction of oscillation at the breakdown.

When the inner wall rotates at a uniform speed (outer wall stationary), the critical rotational speed increases with the axial speed of flow; and the critical number for no axial flow, predicted by extrapolation of the curve drawn through the numbers measured with axial flow, is in close agreement with Taylor's theoretical number.

It is shown that the early breakdown of laminar flow associated with intense entry disturbances can be caused by very weak entry disturbances, provided they are in the form of discrete eddies.

The formation of mercury molecules. II.

By F. L. ARNOT and M. B. McEWEN. (*Communicated by O. W. Richardson, F.R.S.—Received* 17 *December* 1937.)

An investigation of the formation of ionized mercury molecules has been made by the balanced space charge method. The results obtained in this work, together with those previously obtained with a mass spectrograph by Arnot and Milligan, show that molecular ions are formed by the three processes

$$Hg'(2\,{}^3P_1) + Hg'(2\,{}^3P_0) \rightarrow Hg_2^+ + \epsilon \qquad (1)$$

$$Hg'(4\,{}^1P_1) + Hg \rightarrow Hg_2^+ + \epsilon \qquad (2)$$

$$Hg'(5\,{}^3P) + Hg \rightarrow Hg_2^+ + \epsilon. \qquad (3)$$

Ions formed by each of these processes have been detected. The appearance potentials for these three processes are respectively 4·86 and 9·722 V for (1) and (2) and 9·792, 9·796, 9·817 V for the triplet process (3). Ions formed by process (1) which requires a collision between two excited atoms were detected at a vapour pressure of 0·1 mm. of Hg at 0° C. Ions formed by processes (2) and (3) which involve a collision between one excited atom and a normal atom were detected at pressures of the order of 0·01 mm.

The ionization potential of the mercury molecule lies between $9{\cdot}52+D$ and $9{\cdot}32+D$, where D is the work of dissociation of the normal molecule. Using Winan's value for D the limits are 9·67 and 9·47 V.

Excited atoms in states other than P states do not apparently form ionized molecules by attachment even though they have more than sufficient energy to do so.

On the occurrence of helium in beryls. By J. W. J. Fay, E. Glückauf and F. A. Paneth. (*Communicated by J. C. Philip, F.R.S.—Received* 17 *December* 1937.)

Various specimens of old beryllium metal have been analysed for helium. As the method employed, in spite of its sensitivity, failed to detect any traces of helium it must be concluded that the spontaneous production of helium in beryllium is less than $1{\cdot}3\times10^{-11}$ c.c. of helium per gram beryllium per year.

From this figure it follows that the helium content of beryls cannot be explained as a consequence of the spontaneous disintegration of a beryllium isotope of mass eight. Even the assumption that such a beryllium isotope has been present in previous geological periods and now mostly decayed is not compatible with the very low limit found for present-day helium production.

In recent years the helium content of beryls has been attributed to the influence of γ-rays from radioactive minerals in the neighbourhood of the beryls, and to cosmic radiation. From the figures obtained by measurement of the amount of helium produced in beryllium by γ-rays it follows that the influence of the natural sources of γ-radiation is not nearly sufficient to explain the helium content of beryls.

Since, therefore, beryllium does not produce adequate amounts of helium, either under the influence of external radiation or as a consequence of spontaneous disintegration, it seems that the helium content of beryls is not connected with its beryllium content at all, but is due to some other chemical element.

Identification and measurement of helium formed in beryllium by γ-rays. By E. Glückauf and F. A. Paneth. (*Communicated by J. C. Philip, F.R.S.—Received* 17 *December* 1937.)

While it has been known for some time that the beryllium nucleus, irradiated by γ-rays, emits neutrons, it could not be decided whether the nucleus is thereby transformed into a stable isotope of beryllium of mass eight, or into two helium atoms.

By a micro-chemical method helium was detected in beryllium after irradiation by the γ-rays of radon. In one of the experiments the number of neutrons simultaneously emitted during the irradiation was determined by measuring the helium produced by these neutrons in methyl borate; a comparison of the two helium quantities showed that the main final product of the γ-irradiation of beryllium is helium and not the beryllium isotope.

The nature of the penetrating component of cosmic rays. By P. M. S. BLACKETT, F.R.S. (*Received* 18 *December* 1937.)

Further measurements of the energy loss of cosmic rays in metal plates have confirmed the former result of Blackett and Wilson that nearly all the rays with energy less than 2×10^8 e-volts are electronic in character. For higher energies the mean loss is much smaller. A reinterpretation of the data for these higher energies in the light of the cascade theory of showers has confirmed the conclusion of Neddermeyer and Anderson that there must be some energetic but absorbable rays which can be identified with fully radiating electrons. But the number of these rays is quite small, not more than 1 % of the rays at sea level with energy over 3×10^8 e-volts being electrons.

Both direct and indirect evidence is found which shows that the energetic penetrating rays actually become, as they slow down, the absorbable rays of low energy which are indistinguishable from electrons. The main requirement of a theory of the penetrating component is to explain this striking property. The two most obvious types of explanation are as follows. The rays can be considered as heavy when energetic, but to change their mass suddenly when their energy falls below the critical energy. Or, the rays are assumed to have electronic rest mass, but to be distinguished from normal electrons by some new property, which has the effect of making their energy loss vary with their energy and the nature of the absorber.

Oscillography of adsorption phenomena. 3. Rates of deposition of oxygen upon tungsten. By M. C. JOHNSON and A. F. HENSON. (*Communicated by A. M. Tyndall, F.R.S.—Received* 18 *December* 1937.)

An experiment is devised for investigating the passage of small and reproducible quantities of oxygen over a hot tungsten filament whose surface purity can be ascertained by observation of its thermionic emission immediately before each admission of gas. The oxygen pressure at the filament is made to rise within a fraction of a second from a high vacuum to a transient value with a flat maximum of 10^{-3} to 10^{-4} mm. This enables the rate of primary adsorption to be isolated, and its dependence on gas pressure and on temperature to be determined, without the resulting monolayer becoming sufficiently dense to allow oxidation and appreciable thinning of the filament. The adsorption is traced by photographing the accompanying fall of electron emission by means of the oscillographic technique described in previous papers. Within a range of temperature and pressure such that evaporation is negligible and interaction between adsorbed particles is rare, the uncovered fraction of the surface decreases exponentially with time over a portion of each experiment. This enables a condensation constant to be obtained and its temperature dependence evaluated. The temperature coefficient which is found implies a heat of activation of 24,000 ± 3000 cal. per mol. in the vicinity of 2200° abs. The bearing on theories of activated adsorption is briefly discussed.

The law of error and the combination of observations. By H. JEFFREYS, F.R.S. (*Received* 18 *December* 1937.)

The limitations of the theoretical grounds for the normal law of errors of observation are discussed, and seven series of observations capable of providing tests of the law are examined. It is found that the χ^2 test, as usually employed, is not sufficiently sensitive to establish departures from the normal law. A wider grouping, however, reduces the random error of χ^2 enough to show them clearly, though it is still less sensitive than the ratio of the maximum likelihood solution for the departure to its standard error. It appears that no form of the test is of much use when the law to be tested implies very small expectations in some of the groups.

An approximation to the method of maximum likelihood for Pearson laws of types II and VII is developed, and extensions to types I and IV are suggested. The approximation does not require excessive labour or the retention of a large number of figures. It is found that the various series of data give laws ranging from type II with index 4·5 to type VII with index 4·3, and that the index is closely correlated with the degree of correlation of the errors within groups of successive observations. An extrapolation using this correlation suggests that genuinely independent observations would follow a type VII law with index between 3 and 4. Methods of combining observations derived from such a law and determining their uncertainties are provided. It appears that a number of discrepancies in physics and astronomy that have been accepted as systematic may turn out to be random, since with such a law large random errors may occur more often than with the normal law, if the mean and the mean square deviation are still used as estimates.

The crystalline structure of steel at fracture. By H. J. GOUGH, F.R.S. and W. A. WOOD. (*Received* 20 *December* 1937.)

In a previous investigation, precise methods of X-ray diffraction were employed to study the effects on the crystalline structure of normalized mild steel of deformation and fracture under static and fatigue stresses. It was established that failure by static and cyclic stressing was characterized by exactly the same kind of progressive deterioration of the original crystals leading to a completely fragmented system of crystallites of random orientation. In addition to this state, it was considered that the presence of marked lattice distortion in the crystallites was also a necessary condition for fracture but direct and indisputable evidence on this aspect could not be obtained under the conditions of the previous experiments. The further work now described was, therefore, undertaken. A mild steel *initially* in a heavily cold worked condition has been used, enabling the process of further deformation and fracture to be studied: the fatigue method of stressing has been employed in view of the unique advantages offered by it.

While it is found that under safe ranges of stress, no measurable change occurs in the structure, cycles of an unsafe range produce a progressive effect on the efficiency of scattering of the X-rays only to be accounted for by the incidence of a heavy lattice distortion in the crystallites forming the fragmented material. The establishment of this condition of severe internal stress at the fracture stage is the most important result of the work. In addition, however, informative work has also been done on *normalized* mild steel using an improved X-ray technique: among other results, it is shown that the rate of modification of structure, in a fatigue test, decreases as the test proceeds tending to the establishment of a stable structural condition.

Studies on chromatic behaviour in crustacea. The receptive mechanism of the background response. By H. G. Smith. (*Communicated by L. Hogben, F.R.S.—Received* 21 *December* 1937.)

The pigmentary effector system of *Ligia* consists of melanophores and xanthophores separate in some regions and in other regions superimposed so as to form complex chromatophores. The melanophores are the chief agents of visible colour change and their response to illumination is partly direct and partly controlled by the eyes. The primary response is of the type described for decapods and mysids by Keeble and Gamble and for vertebrates by Laurens and Hogben. Intense illumination increases the diffusion of the pigment. The secondary or visual response to background is also of the type described for *Macromysis* and vertebrates. Overhead illumination in a black container makes the animal dark, and overhead illumination of an animal in a white container makes it pale. Blinded animals are not so dark as seeing animals kept on a black background. In darkness they are still more pale owing to elimination of the primary response. The time relations of the background response show that it is controlled by two hormones. The existence of a "supernormal phase" in the transition from white background equilibrium reinforces this conclusion. So also do experiments which show how the supernormal phase may be accentuated or eliminated. The effect of varying (*a*) the direction of incident illumination, (*b*) limiting the light scattered on to the eye make it possible to distinguish two groups of ocelli. One (dorsal) is accessible to direct overhead illumination and is responsible for initiating the discharge of the hormone which evokes melanophore expansion. The other (latero-ventral) picks up light scattered from the immediate surroundings and is responsible for initiating the discharge of the hormone which evokes melanophore contraction. The effects of painting the whole eye, the ocelli of the upper half only, or the ocelli of the lower half alone, directly confirm this conclusion. The blue end of the spectrum is most effective for evoking the visual or secondary response.

On the chromatic behaviour of elasmobranchs. By H. Waring. (*Communicated by L. Hogben, F.R.S.—Received* 21 *December* 1937.)

With few exceptions, all Elasmobranchs so far investigated pale on a white background and darken on a black one. These changes are brought about by the contraction and expansion of dermal and epidermal melanophores. The expanded condition is due to a blood circulated hormone B produced by the neuro-intermediate lobe of the pituitary. Parker and his associates believe that pallor is brought about by the stimulation of melanophoric nerves. The present paper describes two classes of experiments which show that the anterior lobe produces a hormone which antagonizes the B substance produced by the neuro-intermediate lobe. These are: (*a*) effects of removal of the anterior lobe; (*b*) differential tolerance to B-containing extracts of normal animals, animals from which the whole gland has been removed, animals deprived of the neuro-intermediate lobe alone. Reasons are advanced for believing that the class of experiments upon which Parker's interpretation is based is incapable of yielding unequivocal evidence in favour of it.

On the neutrino theory of light. By M. H. L. Pryce. (*Communicated by P. A. M. Dirac, F.R.S.—Received* 21 *December* 1937.)

This paper brings to light a grave difficulty for the neutrino theory of light. Starting from assumptions about the neutrino sufficiently general to include the models which have been studied by Jordan Kronig and others (with the exception of Scherzer's attempt, which is not strictly a neutrino theory), and working with the amplitudes of the second quantization as the most suitable mathematical apparatus, one sets up the most general theory consistent with Jordan's hypothesis. The conditions under which this will lead to a satisfactory theory of light are (1) that certain commutation rules be satisfied; (2) that the theory be invariant under a change of co-ordinate system. In order to study the second of these it has been necessary to analyse rather carefully the transformation of the amplitudes under certain types of rotation and this reveals an arbitrariness in the choice of certain phases. A condition for the invariance of the theory is that the results be independent of the way in which these phases are chosen.

From this point onward a straightforward analysis leads to the result that the conditions cannot be satisfied simultaneously. The invariance requires that the neutrino which interacts with the atom should reverse its spin, a result which could also be derived from considerations of the conservation of angular momentum, and an essentially simple though rather tedious calculation shows this to be inconsistent with the commutation rules.

The introduction gives an account of the aims of the neutrino theory of light, the problems which it meets and the attempts that have been made to solve them.

The absorption spectrum of sulphur dioxide and carbon dioxide in the vacuum ultraviolet.

By W. C. PRICE and D. M. SIMPSON. (*Communicated by O. W. Richardson, F.R.S.—Received* 23 *December* 1937.)

The absorption spectra of sulphur dioxide and carbon disulphide have been investigated by means of a vacuum spectrograph down to 1000 A. For both molecules the systems of bands can be divided into two classes: (1) those which exhibit wide vibrational structure, (2) those which exhibit little or no vibrational structure. The former class probably correspond to transitions to anti-bonding molecular orbitals, while the latter are due to the transitions of comparatively non-bonding electrons to excited orbitals, which are mainly atomic in character. In the case of sulphur dioxide, the extrapolation of the bands of class (2) to their limit gives a value of $12{\cdot}05 \pm 0{\cdot}05$ V. for the ionization potential of the molecule. A similar procedure for carbon disulphide yields the much more accurate values 10·083 and 10·027 V for ionization to the two components of the doublet state $^2\Pi_g$ of CS_2^+ ($\Delta\nu = 436$ cm.$^{-1}$). The experimental evidence indicates that while carbon disulphide is slightly bent in the earlier stages of the excitation, it finally returns to a linear configuration in CS_2^+. A vibrational analysis of the bands of class (1) is also given, and some general features of the electronic spectra of polyatomic molecules are discussed.

Significance tests when several degrees of freedom arise simultaneously.

By H. JEFFREYS, F.R.S. (*Received* 29 *December* 1937.)

Tests are provided for the significance of an estimated departure from a uniform distribution of chance, and of the coefficients of new functions introduced into an empirical law designed to fit a series of measures; in each case where several degrees of freedom may be expected to arise together if one of them does. A test is also given for the independence of errors of observation when the means of groups of consecutive observations are compared with the standard deviation of the entire set. Applications are made to the secular perturbations of the inner planets and to Pearson's data for errors of observation.

Vacuum wavelength measurements in the iron spectrum by means of the reflexion echelon grating.

By W. E. WILLIAMS and A. MIDDLETON. (*Communicated by O. W. Richardson, F.R.S.—Received* 29 *December* 1937.)

The vacuum wave-lengths of forty-seven iron lines have been determined directly against the red cadmium standard by means of a new method employing a reflexion echelon in vacuum so that no corrections for the refractive index of normal air need be made. By using a Schüler type hollow cathode source, much narrower lines are obtained than is possible with an iron arc and the concordance of independent determinations justify the values being given to eight significant figures. The work is being extended further to the ultra-violet.

The forces on a cylinder, or flat plate, submerged in a uniform stream.

By T. H. Havelock, F.R.S. (*Received* 29 *December* 1937.)

General expressions are obtained for the resistance, lift and moment for a cylinder submerged in a uniform stream, including circulation, the analysis being an extension of that used in a previous paper on the circular cylinder. Application is made to the elliptic cylinder, the solution involving an integral equation which may be solved by successive substitution to any required degree of approximation.

Special consideration is given to the flat plate, which is treated as the limiting case of an elliptic cylinder; expansions are obtained for the resistance, lift and moment, and graphs of the two latter quantities are given.

The general expressions include, as special cases when gravity is neglected, the problem of a flat plate in a uniform stream bounded by a free surface or by a plane wall; these cases are examined with reference to the interference of the surface of the sea or of the ground upon the lift and moment of an aerofoil.

Collective electron ferromagnetism.

By E. C. Stoner. (*Communicated by O. W. Richardson, F.R.S.—Received* 29 *December* 1937.)

General equations are obtained, using Fermi-Dirac statistics, for the magnetic moment, M, of a number, N, of electrons in an unfilled energy band, for which the interchange interaction effects give rise to a term in the energy expression proportional to the square of the magnetization. The relative magnetization, $\zeta(=M/N\mu)$, is expressible as an implicit function of the reduced field, $\mu H\epsilon_0$, temperature, kT/ϵ_0, and interaction energy coefficient, $k\theta'/\epsilon_0$, where ϵ_0 is the Fermi energy without interaction. Particular limits correspond to equations for electron spin paramagnetism, and to the Weiss-Heisenberg "classical" equations for ferro- and paramagnetism.

The equations are solved numerically, using tables of Fermi-Dirac functions (McDougall and Stoner), in such a way as to give kT/ϵ_0 as a function of ζ for a range of values of $k\theta'/\epsilon_0$. The results are shown in a series of tables and diagrams.

The character of the dependence of ζ on kT/ϵ_0, or on T/θ, depends on the ratio $k\theta'/\epsilon_0$. A necessary condition for ferromagnetism is $k\theta'/\epsilon_0 > \frac{2}{3}$, while for $k\theta'/\epsilon_0 < 2^{-\frac{1}{3}}$ $(=0{\cdot}793701)$, the relative magnetization at absolute zero, ζ_0, is less than unity. For small values of ζ_0, the magnetization-temperature curve is closely represented by

$$(\zeta/\zeta_0)^2 = 1-(T/\theta)^2,$$

but the curve does not change monotonically to the classical form as $k\theta'/\epsilon_0$ increases.

Series expansions appropriate for particular ranges are given for $k\theta'/\epsilon_0$ as a function of $k\theta/\epsilon_0$, and of kT/ϵ_0 and ζ; and expressions are derived for the variation of the magnetization near the Curie point and at low temperatures.

A full discussion of the experimental results is deferred, but it is shown that the lack of agreement between the values deduced for the saturation moment from the paramagnetism above the Curie point and from the low temperature magnetization receives an immediate interpretation.

A new basis for cosmology. By P. A. M. Dirac, F.R.S. (*Received* 29 *December* 1937.)

It is proposed that all the very large dimensionless numbers which can be constructed from the important natural constants of cosmology and atomic theory are connected by simple mathematical relations involving coefficients of the order of magnitude unity. The main consequences of this assumption are investigated and it is found that a satisfactory theory of cosmology can be built up from it.

The polarization of a calomel electrode. By Lord Rothschild. (*Communicated by J. Gray, F.R.S.—Received* 30 *December* 1937.)

The maximum current density which an anodic calomel electrode will sustain over reasonable periods of time (5 hr.) without polarization is 15 amp.$^{-6}$ cm.$^{-2}$.

Cytochrome oxidase. By D. Keilin, F.R.S. and E. F. Hartree. (*Received* 30 *December* 1937.)

1. The addition of 10^{-5} to 10^{-4} M cytochrome *c* to heart muscle oxidase preparation greatly increases the rate of catalytic oxidation of a number of diamines and polyphenols such as *p*-phenylenediamine, *p*-aminophenol, hydroquinone, adrenaline, catechol, etc.
2. All these compounds readily reduce a solution of oxidized cytochrome *c*.
3. The rate of oxidation of these compounds by oxidase preparation may increase, on addition of cytochrome *c*, up to 30–40 times.
4. The much slower oxidation of these compounds without addition of cytochrome *c* is due to cytochrome already present in the oxidase preparation.
5. The oxidation of all these compounds therefore is not catalysed directly by indophenol oxidase but through co-operation with cytochrome. In other words, the indophenol reaction of cells and their extracts takes place through cytochrome.
6. The only catalytic property which can be definitely ascribed to the muscle oxidase (known as indophenol oxidase) is the oxidation of reduced cytochrome. The correct name for this enzyme should, therefore, be *cytochrome oxidase*.
7. The explanations as to the nature, distribution and catalytic activity of this enzyme proposed by Remesow, Bigwood, Harrison and Shibata with his co-workers, are discussed and shown to be incorrect.
8. There is no necessity to assume the existence in the respiratory system of the cell of a component undergoing reversible "oxygenation" as was proposed by Shibata and his co-workers.

Relation between the uterus and the ovaries in the pregnant hamster.

By M. Klein. (*Communicated by A. S. Parkes, F.R.S.—Received* 3 *January* 1938).

1. Experiments on the physiology of pregnancy have been carried out on the golden hamster.
2. Complete ovariectomy performed between the 9th and the 13th day results in the termination of pregnancy.
3. The injection of progesterone alone failed to maintain pregnancy and the typical vaginal mucification in hamsters ovariectomized about the 10th day after mating. The simultaneous injection of progesterone and oestrone did, however, maintain pregnancy and vaginal mucification in ovariectomized females, and in several cases we have obtained parturition at term with living litters. These results suggest that, in the hamster, progesterone and oestrone may exercise endocrine control of pregnancy.
4. Complete removal of the pregnant uterus between the 8th and the 13th day causes the premature and rapid regression of the corpora lutea of pregnancy, and the ovarian cycle restarts. When the foetuses only are removed, by caesarian section, and the placentae remain inserted, the corpora lutea of pregnancy are maintained and inhibition of the ovarian cycle continues.
5. These results agree with and complete our previous experiments on the rabbit and the rat. They show that there is a close correlation between the pregnant uterus and the ovaries, though we cannot yet postulate a satisfactory interpretation of the mechanism of the connexion.

Progressive lightning. V. A comparison of photographic and electrical studies of the discharge process.

By B. F. J. Schonland, D. B. Hodges and H. Collens. (*Communicated by Sir Charles Boys, F.R.S.—Received* 5 *January* 1938.)

A detailed comparison is made of the results of Boys' camera observations of the luminosity changes during the lightning discharge and of observations with a cathode ray oscillograph of the electric field. In certain cases these observations were simultaneous.

The polarity of the discharges to ground was found to be negative in all cases examined.

The results confirm and extend the identifications of the electric field changes made by Appleton and Chapman. During a ground stroke these begin with the slow lowering along the leader channel of about 60 % of the cloud charge tapped, and are followed by the sudden removal of this leader charge to ground by the return streamer and by the slower passage of the remainder of the cloud charge to ground.

The more important transient induction and radiation effects are associated with the stepped leader to first strokes and with the start of the return streamer.

Since the return streamer is absent in most discharges within the cloud it is suggested that discharges to ground are the chief cause of atmospherics from distant thunderclouds.

A suggestion for unifying quantum theory and relativity. By M. BORN.

(*Communicated by E. T. Whittaker, F.R.S.—Received* 5 *January* 1938.)

The fact that the fundamental laws of quantum mechanics are symmetrical in space-time x^k and momentum-energy p_k can be generalized to a "principle of reciprocity" according to which the x space and the p space are subject to geometrical laws of the same structure, namely a Riemannian metric. In analogy with Einstein's closed x world one has to assume that energetically closed systems (as elementary particles, nuclei) must be described by help of a hyperspherical p space. A consequence of this assumption is a modification of the formula for the number of quantum states in an element of the p space. The application of this formula to quantum electrodynamics leads to a finite zero energy of the vacuum, a finite self-energy of the electron, etc. Deviations from Planck's law and the Stephan-Boltzmann law of radiation, and the caloric properties of gases are predicted for very high temperatures.

X-ray studies of the structure of hair, wool and related fibres. IV. The molecular structure and elastic properties of the biological cells. By H. J. WOODS.

(*Communicated by Sir William Bragg, O.M., P.R.S.—Received* 5 *January* 1938.)

From a study of the cells isolated from various types of animal hair it is concluded that they contain crystalline keratin, and when the cells are long, tissues can be built up to give X-ray photographs closely comparable with those of the fibres. Cells obtained by retting fibres previously set in steam are found to be stretched correspondingly, although their measured lengths after retting are somewhat shorter than would be expected. This lag is associated more with the early stages of extension, indicating that the elastic phase K_1 extends inside the cells, as well as between them. The X-ray photograph of elongated cells from fibres set at high extensions is the ordinary one of β-keratin. The elongated cells only recover partially when they are boiled in water, and the permanent set remaining is of the same order as that of fibres steamed for the same time at the same extension as those from which the cells were obtained. Permanent set is thus also a property of the cells themselves.

Cotswold wool fibres which have been relaxed in dilute caustic soda can also be disintegrated into cells, which recover during the process to rather less than the normal length. These relaxed cells can be supercontracted still further by boiling water. In this way cells of about half the normal length may be obtained; their crystalline part shows itself in the β-form, just as does that of highly supercontracted fibres.

By the combined action of lateral pressure and steam the cells from normal fibres can be compounded into coherent transparent sheets which are elastic in cold water for extensions up to about 50 % and extensible in steam or caustic soda by twice this amount. These sheets can be relaxed, set, and supercontracted, in the same way as fibres, and their tensile strength in water is about 25 % of that of the fibres. It is suggested that the development of coherence during the treatment in steam is of a similar type to the development of permanent set in the stretched fibre.

Since such differences as exist between the properties of the cells and those of the fibres are only of the same order as those which occur between various types of hairs, it is clear that the behaviour of the fibres is for the most part due to the cells. The histological heterogeneity of hairs can affect only the details of their behaviour.

Keratin, stretched denatured edestin, and stretched myosin are all anisotropic in their electrical properties, having different dielectric constants for fields parallel and perpendicular to the protein chains. By hanging discs of these proteins in an alternating electric field it is therefore possible to determine the direction of the chains.

Polyphenol oxidase. Purification, nature and properties. By D. KEILIN, F.R.S. and T. MANN. (*Received* 11 *January* 1938.)

1. A comparatively simple method of preparation of highly purified polyphenol oxidase from cultivated mushrooms (*Agaricus campestris*) is described.

2. The activity of the enzyme in different preparations was determined by two methods: (1) manometric, from the initial rate of oxygen uptake by catechol at 20° C., calculated per mg. dry weight per hour and expressed as Q_{O_2}, and (2) colorimetric, from milligrams of purpurogalline formed from pyrogallol in 5 min. at 20° C. per mg. dry weight and expressed as purpurogalline number (P.N.). The amount of enzyme which, under these conditions produces 1000 mg. purpurogalline is taken as one enzyme unit (E.U.).

3. The purest enzyme preparation obtained was completely free from haematin and contained only traces of iron and manganese.

4. The polyphenol oxidase of mushrooms was found to be a copper-protein compound, the copper being a constituent of the active group of the enzyme. This extends the discovery of Kubowitz, who found that the corresponding oxidase in potatoes is a compound of copper with protein.

5. No proportionality between the copper content and the enzyme activity was found for preparations ranging from P.N. = 0·45 to P.N. = 250. These preparations contain large amounts of copper which does not belong to the enzyme. Within this range the amount of copper per enzyme unit (E.U.) gradually diminishes from 196γ to 7γ. Only when the copper content reaches a low and constant value of about 3·2–3·5γ per E.U. does the copper content become strictly proportional to the enzyme activity.

6. The purest enzyme preparation obtained has P.N. = 940, Q_{O_2} = 1,160,000 and copper content of 0·30 %. As this copper content is higher than that of pure crystalline haemocyanine, which may vary according to origin, from 0·173 to 0·26 % (Hernler and Philippi), the enzyme may be considered as pure. 1γ Cu of this enzyme transfers to catechol, at 20° C. in 1 min., about 6000 c.mm. O_2. 9·6 mg. of this enzyme (dry weight) was obtained from 15 kg. fresh mushrooms.

7. The crude extract of mushrooms readily oxidises only such compounds as catechol, pyrogallol and *p*-cresol. Other related compounds such as hydroquinone and *p*-phenylenediamine are hardly oxidised by this enzyme.

On purification, the enzyme gradually loses the property of catalysing the oxidation of monophenols.

8. All the oxidation reactions catalysed directly or indirectly by the polyphenol oxidase are inhibited by KCN, H_2S and CO.

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable. The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form. These abstracts will be issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings".

The influence of radiation on ionization equilibrium. By B. SRIVASTARA. (*Communicated by M. N. Saha, F.R.S.—Received* 11 *January* 1938.)

The generalized ionization formula in the case of thermodynamical equilibrium for an assembly consisting of atoms, ions, electrons and radiation is derived, the particles being supposed to obey any statistics.

The ionization produced in matter has been found for the case when the reaction space is traversed by radiation at a higher temperature. The electrons have been assumed to obey F.D. statistics, while the atom and the ion are taken to obey Boltzmann (classical) statistics. The general ionization formula for this case is derived and two limiting cases discussed. The Woltjer-Milne formula for ionization in any radiation field is readily obtained as a special case of the general formula. The ionization formula for the case when the electrons behave as a degenerate gas is also deduced from the general formula. On substituting the usual expression for the probability of ionization, viz. $\psi = C\nu^{-3}$, it is found that the ionization becomes much less when the electrons are highly degenerate.

Uranium Z and the problem of nuclear isomerism. By N. Feather and E. Bretscher. (*Communicated by E. V. Appleton, F.R.S.—Received* 13 *January* 1938.)

The radiations from uranium Z have been examined by the absorption method using a tube counter. The effective quantum energy of the γ radiation is $0{\cdot}70 \pm 0{\cdot}05 \times 10^6$ e-volts and the intensity $1{\cdot}50 \pm 0{\cdot}25$ quanta per disintegration. An analysis of the β radiation into continuous spectra with limiting energies 0·56 and $1{\cdot}55 \times 10^6$ e-volts, and intensities in the ratio 17 : 1, is suggested, though it is pointed out that the component of lower energy is probably itself complex. The uranium X_2-uranium Z branching ratio has been determined as $665 \pm 65 : 1$.

On the basis of these results the isomerism of the nuclei UX_2 and UZ is discussed in the light of v. Weizsäcker's hypothesis, and a level scheme is put forward which appears to account for all the facts. Reasons are given in support of the conclusion that uranium Z is formed from uranium X_2 in a β-γ branching, rather than from uranium X_1, directly, in a β-β transformation.

On the primary decomposition of ethane and the reaction between ethane and nitric oxide. By T. J. Gray, M. W. Travers, F.R.S. and F. T. White. (*Received* 14 *January* 1938.)

The thermal decomposition of pure ethane, and of a mixture of ethane and nitric oxide, have been studied at 590° in silica apparatus by the method of detailed analyses.

The observation of Staveley (1937) that the addition of nitric oxide to ethane reduces the rate of primary decomposition is confirmed. However, it is shown that a rapid reaction takes place between ethane and nitric oxide, without apparent increase in volume, which accounts for the fact that it was not observed by the pressure measurement method which Staveley employed. The product of the ethane-nitric oxide reaction at first increases in concentration in the gas and then diminishes. It has not been isolated.

The ethane-nitric oxide reaction itself slows down after an interval, but as it does so a reaction which appears to involve ethane and the product of the ethane-nitric oxide reaction develops. One product of this reaction appears to be ethylene.

The theory of free radicals cannot be used to explain the facts now put forward.

It is suggested that the ethane-nitric oxide reaction operating with a lower activation energy than the primary decomposition process, removes *hot* molecules at a rate faster than they can be supplied to maintain the Maxwell-Boltzmann equilibrium. The result is that the former process is to a considerable extent *substituted* for the latter. The ethane-nitric oxide reaction in turn slows down at a rate faster than can be accounted for by the removal of ethane or nitric oxide. This is associated with the operation of a reaction between ethane and a product of the ethane-nitric oxide reaction at an activation energy still lower than the ethane-nitric oxide reaction itself.

A method of photographing airscrew sound-waves. By W. F. Hilton. (*Communicated by L. Bairstow, F.R.S.—Received* 15 *January* 1938.)

An experimental technique has been developed for photographing the shock waves generated by an airscrew at all speeds above a certain critical speed. A modified form of spark photography was employed, and photographs were obtained of the airscrew sound-wave system at top-speeds ranging from 0·83 to 1·2 of the velocity of sound.

The photographs also reveal a trailing vortex from the blade tip, and an eddying wake behind the blade. These vortices and eddies are more distinct at high speeds than at low, but there is no definite lower limit to the speed at which they can be photographed.

A method is advanced for calculating the pressure amplitude of the shock waves from measurements of photographs, and also for calculating the speed at which a low wave can first appear in front of the blade. The wave velocity has been calculated from the photographs, and is found to be slightly greater than that of small amplitude sound waves.

A method of calculating the shape of the waves is examined, but no indication is given of a wave front at tip speeds less than that of sound, whereas a very definite wave front exists down to tip speeds as low as 0·85 of sound velocity.

The problem of noise reduction in aircraft is discussed.

The crystal structure and magnetic properties of $CuSO_4.5H_2O$. By K. S. Krishnan and A. Mookherji. (*Communicated by Sir Venkata Raman, F.R.S.—Received* 18 *January* 1938.)

The crystal structure of $CuSO_4.5H_2O$ has been analysed by X-ray methods by Beevers and Lipson. On the basis of the structure proposed by them, and the theory developed by Van Vleck and others which attributes the magnetic anisotropy of a paramagnetic crystal to the asymmetry of the strong electric fields acting on the paramagnetic ions in the crystal, the axes of the magnetic ellipsoid of the crystal, and its eccentricity, are deduced theoretically.

Detailed experimental data are given for the setting-directions and the magnetic anisotropy for several planes in the crystal. The axes of the magnetic ellipsoid, and the orientations of the central circular sections of the ellipsoid (which give a measure of the relative magnitudes of the principal diameters of the ellipsoid), are determined from the magnetic data. They agree well with the results deduced from the structure.

The magnetic anisotropy of the ultimate paramagnetic anisotropic units, consisting each of a Cu^{++} ion and six negatively charged oxygen atoms surrounding it, which form a regular octohedron with one of its diagonals elongated, are calculated from the magnetic and the structural data for the crystal. These paramagnetic unit groups are approximately tetragonal in symmetry, the susceptibility along the tetragonal axis being greater than for perpendicular directions by about 550×10^{-6} per g. ion of Cu^{++}. This anisotropy gives us a measure of the asymmetry of the electric fields acting on the Cu^{++} ions in the crystal.

Diamagnetism of cadmium. By S. RAMACHANDRA RAO and S. SRIRAMAN.

(*Communicated by O. W. Richardson, F.R.S.—Received* 18 *January* 1938.)

Single crystals of cadmium were prepared by the method of slow cooling and the principal magnetic susceptibilities were determined by the Guoy method. The influence of ferromagnetic impurities on the observed values was eliminated by determining the susceptibilities at different field strengths in accordance with Honda's method. The mean values perpendicular and parallel to the hexagonal axis of the crystal were found to be $-0{\cdot}163$ and $-0{\cdot}223$ respectively. The magnetic anisotropy was $1{\cdot}368$ and the mean diamagnetic susceptibility for a polycrystalline aggregate $-0{\cdot}183$. The value $-0{\cdot}163$ of $\chi_\perp$ is close to that of McLennan, Ruedy and Cohen but our value of $\chi_\parallel$ is lower than theirs. This makes the magnetic anisotropy of cadmium much nearer that of zinc than the earlier values indicated.

The influence of cold-working on polycrystalline rods and single crystals of cadmium was investigated. In the case of polycrystalline specimens, a small decrease was obtained. On the other hand, the principal susceptibilities measured with single crystals showed no variation on cold-working.

The influence of small quantities of foreign matter was also a subject of investigation. Lead when added in small amounts affected the principal susceptibility normal to the *c*-axis but not the value parallel to this axis. The principal susceptibilities did not alter as a result of adding small quantities of zinc.

These results are examined in the light of the theory of paramagnetism of free electrons developed by Pauli, Landau and Peierls. The valency electrons appear to contribute a paramagnetic component perpendicular to the c-axis. There is evidence for this from electrical conductivity data. The results obtained with the alloys are examined in the light of these conclusions.

Tests of statistical hypotheses which are unbiased in the limit. By J. NEYMAN.

(*Communicated by G. U. Yule, F.R.S.—Received* 19 *January* 1938.)

Propositions I and II show that the conception of a test which is unbiased in the limit, first introduced in connexion with the "smooth" test for goodness of fit, is applicable to a great variety of problems, where technical difficulties prohibit the application of unbiased tests of type A. Propositions III and IV provide the possibility of determining approximately the power functions by calculating their limits, for fixed values of the standardized errors in the hypothesis tested and for the number of observations indefinitely increasing. These theoretical results are illustrated on two examples.

Owing to the relative easiness of handling the tests unbiased in the limit promise to be of great use. But it is necessary to remember their limitations: a test "unbiased in the limit" may be biased for any finite number of observations, though the actual bias is probably frequently negligible. We must remember further that the Propositions III and IV give us only approximate information concerning the power function and, what is more, the degree of the approximation is not known. It follows that some further research in the matter is desirable.

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18 February 1938

The probability of annihilation of positrons without emission of radiation. By H. S. W. Massey and E. H. S. Burhop. (*Communicated by R. H. Fowler, F.R.S.—Received* 19 *January* 1938.)

When mutual annihilation occurs on collision of an atomic electron and a positron the energy liberated, instead of appearing as radiation, may be absorbed by a second atomic electron, resulting in its ejection from the atom. In this paper the probability cross-section for this radiationless annihilation process, involving the K-electrons of a lead atom, is calculated with accuracy, account being taken of the repulsive influence of the nucleus, retardation, spin-spin interaction and electron exchange. Estimates are also made of the contributions from other pairs of electrons and the conclusion is finally reached that the total cross-section for radiationless annihilation by lead electrons attains a maximum value of between 2 and 3×10^{-26} cm.^{2} for positrons with energy 300,000 e-volts. The possibility of observing the phenomenon is briefly discussed.

The adsorption of vapours at plane surfaces of mica. I. By D. H. Bangham and S. Mosallam. (*Communicated by D. L. Chapman, F.R.S.—Received* 20 *January* 1938.)

Measurements have been made of the quantities of benzene, carbon tetrachloride, and methyl alcohol adsorbed at a known surface area of mica at pressures ranging from 0·02 mm. to near saturation. The general shape of the benzene isotherm is sigmoid, convex to the adsorption axis at lower pressures, where the Langmuir monolayer is incomplete, but becoming markedly convex to this axis near saturation, where the film is multimolecular. The same isothermal is obtained when the mica plates are separated by fine wires as when they are packed closely face to face. Capillary condensation of liquid in incipient cleavages at the mica edges does not take place, and the thick films have properties which distinguish them from the bulk liquids. The isotherms of benzene and methyl alcohol at lower pressures agree well with the theoretical equation for films of mobile molecules oriented end-on to the surface, but Langmuir's equation leads to limiting adsorption values for the monolayer which bear no relation to the lattice constants of the mica. The isotherm of carbon tetrachloride shows marked discontinuities, and a determining factor in the adsorption of this substance appears to be the tendency of the molecules to cluster together.

Polish on metals. By W. Cochrane. (*Communicated by G. P. Thomson, F.R.S.—Received* 20 *January* 1938.)

When electron diffraction patterns consisting of diffuse rings are obtained by Thomson's reflexion method, their interpretation is uncertain since such diffuseness may be caused by the geometrical configuration of the surface of the specimen. The pattern of two haloes obtained by previous workers from polished metal surfaces cannot, therefore, be held to prove the existence of an amorphous layer of metal although there is some evidence in favour of this view. In order to eliminate this difficulty, a thin layer of gold was formed on a nickel base. The gold film was polished, then stripped off and examined by transmitted electrons incident normal to the film. In this case the pattern does not depend on the form of the surface of the film. The resulting pattern consisted of three diffuse rings. It was deduced that the film consisted of very small crystal grains and that the atoms in the polish layer of a metal are arranged similarly to the atoms in a monatomic close-packed liquid at a given instant. After a period of 15 hr. the polished gold film yielded a transmission pattern of sharp rings characteristic of polycrystalline gold, showing that crystal growth had taken place with a return to the normal crystal size.

The spectrum of thallium chloride. By H. G. Howell and N. Coulson, (*Communicated by W. E. Curtis, F.R.S.—Received* 22 *January* 1938.)

The spectrum of TlCl has been studied in absorption and also in emission by means of a high frequency discharge. Continua and diffuse bands together with a discrete band system have been found in absorption within the region 2500–3500 A. The band system also occurs in emission. It is considered that the transition is $| \rightleftarrows {}^1\Sigma^+$. the levels having the following constants.

Level	ν	ω_e	$x_e\omega_e$
$\vert$	31054·2	216·91	6·80
${}^1\Sigma^+$	0	287·47	1·24

The analysis of this system given by Butkow is shown to be incorrect. Predissociation occurs in the band system and is probably responsible for the band intensities being the same in both emission and absorption. An attempt to identify the electronic levels of TlCl based upon the similarity of the spectrum to that of TlF has been made.

No sign of the Tl_{203} isotope has been detected whereas the Cl isotope effect completely supports the proposed analysis.

Hyperfine structure, Zeeman effect and isotope shift in the resonance lines of potassium. By D. A. Jackson and H. Kuhn. (*Communicated by F. A. Lindemann, F.R.S.—Received* 24 *January* 1938.)

1. The intensity ratio of the hyperfine structure components of the resonance lines of K 39 was measured by the method of absorption in an atomic beam. The value 1·45 found agrees with the value required by the spin 3/2 if allowance is made for the overlapping by the lines of the 14 times rarer isotope 41. The component of longer wave-length was the stronger, showing that the nuclear magnetic moment of K 39 is positive.

2. By using three atomic beams in series, of collimation 1/35, it was possible to resolve the lines of K 41 as two satellites on either side of the weak component of K 39. The ratio of the splittings of the lines of K 39 and K 41 is found to be 1·77, in good agreement with the value 1·80 found by Manley in magnetic deflexion experiments. The magnetic moment of K 41 is positive, like that of K 39.

The lines of K 41 have an isotope shift of $+0{\cdot}0076$ cm.$^{-1}$ relative to the lines of K 39. The theoretical centre of gravity shift, considering potassium as hydrogen-like, is $+0{\cdot}0087$ cm.$^{-1}$.

3. The Zeeman Effect of the hyperfine structure of the line $4S_{\frac{1}{2}} - 4^2P_{\frac{3}{2}}$ of K 39 was investigated. Each of the two π-components was found to consist of four lines, proving that the nuclear spin of K 39 has the value 3/2, in agreement with magnetic deflexion experiments and the measurements of the intensity ratio of the hyperfine structure lines. The observed positions of the lines are in close agreement with the positions required by the quantum theoretical formulae.

Further investigations of the velocity of propagation of light *in vacuo* in a transverse magnetic field. By C. J. Barnwell and C. C. Farr, F.R.S. (*Received* 25 *January* 1938.)

An experiment designed to determine to a higher degree of precision than before the effect of a transverse magnetic field on the velocity of propagation of light, *in vacuo*, is described. An interferometer method, using the Michelson arrangement, is employed, one interfering ray passing through the field in the gaps of three electromagnets and the other not. A photoelectric cell, and amplifier, is used in conjunction with a galvanometer, to record band movements on a moving photographic strip, the resulting record being subsequently measured by means of a planimeter. By taking the mean of the results from a number of such records, and by carrying out preliminary check experiments, it is considered that all the main causes tending to give a spurious effect greater than an assignable amount have been eliminated. The final experimental results indicate that, in a transverse magnetic field of 20,000 gauss, with residual air at a pressure of 0·05 mm., the velocity of light increases by 32·2 cm./sec., with a probable error of 21 cm./sec. In view of the relatively large probable error, and for other reasons, it is considered that the result may be spurious. All that can be said definitely is that in such a field the effect is less than 1 part in about 5×10^8.

The scattering of cosmic ray particles in metal plates. By P. M. S. Blackett, F.R.S. and J. G. Wilson. (*Received 27 January* 1938.)

Measurements have been made of the multiple scattering of cosmic rays in the following metal plates: 0·33 cm. lead; 1·0 cm. lead; 2·0 cm. copper. The range of values of $H\rho$ of the tracks extended from 10^6 to 3×10^7 gauss-cm., corresponding to electron energies of 3×10^7 to 9×10^9 e-volts.

The observed average angle θ of the multiple scattering is found to be nearly inversely proportional to the measured values of $H\rho$, and to be in fairly close agreement with the prediction of theory for rays of any mass, but with velocity nearly equal to c.

At high energies, the observed values are somewhat higher than expected. This discrepancy, which may be partly due to experimental error, will be further investigated.

This result, that the scattering of the penetrating component is normal, while the radiation loss is much less than that expected for electrons, gives support for a heavier rest mass for the rays. But this conclusion is not quite certain.

The development of the spark discharge. By T. E. Allibone and J. M. Meek. (*Communicated by S. R. Milner, F.R.S.—Received* 31 *January* 1938.)

A general account is given of the development of the high-voltage electric spark, based on the study of over 1000 photographs taken with a rotating film camera.

The electric spark is shown to consist of two principal components, a leader stroke and a main stroke—analogous to the lightning flash. A leader stroke invariably starts from a positive electrode, and sometimes also starts from a negative electrode: sometimes the structure of the leader stroke is simple, sometimes it is of the "stepped" variety. The leader stroke is always branched at many places, and the direction of branching is the direction of its propagation in space: branching thus forms a criterion of the direction of leader-stroke development. The main stroke develops in the reverse direction to that of the leader stroke and at a velocity too high to be recorded. The velocities of the positive and negative leader strokes are of the order of 10^6–10^7 cm./sec., the positive leaders being the faster. Oscillograms of current and voltage support the photographic results. Multiple stroke discharges have been produced and show the characteristics of the multiple lightning flashes; the first is initiated by a slow leader stroke and is branched, the subsequent main strokes are either without leader strokes or the resolution of the camera is inadequate to show them: they are not branched.

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3 MARCH 1938

Investigations of the mechanism of the transmission of plant viruses by insect vectors. II.

By H. H. STOREY.

(*Communicated by F. T. Brooks, F.R.S.—Received* 26 *January* 1938.)

An attempt has been made to determine the conditions that decide whether *Cicadulina mbila* succeeds or fails in transmitting the virus of streak disease of maize. Evidence shows that individual insects, although belonging to an active race and therefore all capable of acting as vectors, vary greatly in their ability to cause infection during a short period of contact with the plant. The results obtained by comparing the infections following contacts of single insects and groups of insects are interpreted as conforming with an hypothesis of independence; that the effect of one insect is independent of the effects of any other insects that may puncture the plant. If this interpretation be correct, a small but definite variation in susceptibility of the maize plants used can be recognised.

By removing at the end of the contact the portion of leaf-tissue exposed to the insect, the probability of infection was somewhat reduced. The effect, though probably significant, was small; and it was concluded that the virus had normally become established in the plant and had moved down at least a few millimetres during the period that the insect maintained contact.

Cicadulina mbila, while resting on a maize leaf, always has its mouthparts inserted, although it may change the position of puncture and may suck material from the leaf only intermittently. The stylets penetrate all tissues of the leaf, but appear to be moved frequently until the phloem is entered.

The insect can take up virus from a chlorotic area of a diseased leaf during a puncture lasting only 15 seconds, which never penetrates beyond the mesophyll. If confined to the green part of the leaf lying between the chlorotic areas, punctures, whether to the mesophyll or to the phloem, fail to take up any virus.

No feature in the behaviour of the insects while in contact with a leaf could be certainly related to their success in causing infection, except that the observations suggested that a high defaecation rate is unfavourable. Evidence was obtained that a single puncture can result in infection. The traces of all such single infective punctures examined entered the phloem; those of unsuccessful punctures sometimes entered the phloem and sometimes ended elsewhere.

The insect can inoculate the virus successfully by inserting its stylets through a wax membrane into a leaf held below, but only if the membrane is not too thick to prevent the stylets reaching the phloem. Seedlings were not infected by insects feeding on the coleoptile; in this organ the phloem is deep-seated, beyond the reach of the stylets.

Plants were never infected by punctures, however numerous, if they were all of less than a certain duration. This threshold-period is about 5 min. at temperatures between 23 and 26° C. A study of the puncture traces showed that many sub-threshold punctures penetrated to the phloem.

A consideration of all the evidence causes me to advance the hypothesis that the insect inoculates the virus in distinct doses, each independent in its effect of any other doses that may be inoculated by the same or other insects. The delivery of a dose is determined by some incident that occurs only after puncture has been maintained for some time.

The influence of rate of deformation on the tensile test with special reference to the yield point in iron and steel. By C. F. Elam. (*Communicated by Sir Harold Carpenter, F.R.S.—Received* 26 *January* 1938.)

Tensile tests on armco iron and two steels have been carried out in a 50 ton Buckton testing machine and in a spring testing machine designed by Quinney. The distortion at the yield point and the formation of Lüder's Lines were investigated in both machines. Tests with these metals and with copper and certain aluminium alloys at different speeds confirmed previous observations that the faster the rate of loading the higher the stress. On the other hand, at very low speeds, there was a greater increase of hardness in the case of iron and mild steel and the aluminium alloys than at faster rates of testing. In copper the reverse was the case. The deformation at the yield point is compared with the "jumping" deformation characteristic of many substances. It is suggested that under certain conditions, the rate of deformation may be greater than the rate of increase in hardness and that rests or very slow rates of extension may equally well increase the effect of work-hardening as allow relaxation to take place.

The resistance of superconducting cylinders in a transverse magnetic field. By A. D. Misener. (*Communicated by J. D. Cockroft, F.R.S.—Received* 31 *January* 1938.)

Detailed experiments on the transition of polycrystalline cylindrical wires from the superconducting to the normal state in a uniform transverse magnetic field are described. Several specimens each of tin, indium and lead of high purity were investigated. The applied field strength (H_t) which restored the first traces of electrical resistance was accurately determined at various temperatures below the normal transition point. It is found that the ratio of this field to the critical field (H_k) corresponding to the temperature of the experiment is not constant but varies with the temperature. The variation is linear, the ratio H_t/H_k increasing as the temperature is decreased. For all specimens the ratio H_t/H_k would have the value 0·50 at the normal transition point.

It is considered that this effect is incompatible with the idea of an "intermediate state" existing in a long cylinder, and an explanation is offered based on the assumption that after penetration of the external magnetic field (above 0·50 H_k) the cylinder breaks up into macroscopic regions of normal and superconducting material. The agreement of this hypothesis with previously observed phenomena is discussed.

A preliminary experiment is reported in which, by measuring the resistance of successive millimeter sections of a specimen, the coexistence of these normal and superconducting regions is shown directly.

β-transitions in a coulomb field. By F. Hoyle. (*Communicated by R. H. Fowler, F.R.S.—Received* 31 *January* 1938.)

This paper attempts to give the selection rules, and the possible forms of the electron energy spectra, which correspond to elements on the first, second, and third Sargent curves, in the case of each of the possible forms of β-interaction belonging to Hamiltonians that contain a derivative of only the neutrino wave function. This allows a choice of several possibilities for the interaction, among which is the form proposed by Konopinski and Uhlenbeck. The accurate solution of the problem would require a knowledge of the wave equation of a nucleus containing many particles. I assume that a non-relativistic Schrödinger equation can be formulated for the nucleus, in which the spin co-ordinate of each particle has two possible values (3, 4). The solutions of such equations for the initial and final nuclei will give a first type of forbidden transition. It is further assumed that a relativistic equation can be constructed for the nuclei, in which the spin co-ordinate of each particle will now have four values (1, 2, 3, 4). From this point of view I regard the Schrödinger equations as given by neglecting, in the relativistic equations, all components of the wave function in which any spin co-ordinate is 1, 2. Generalizing from the Pauli reduction of the Dirac equation in the single-body problem, to the assumption that a component of the wave function in which one spin co-ordinate is different from 3, 4 can be expressed to a suitable approximation, in terms of those components which occur in the Schrödinger equation, the connexion between these components seems to be analogous to that given by Pauli in the one-body problem. This introduces small components of the wave functions into the expression for the transition probability. These small components will have selection rules which are different from those for the large components, and transitions which were forbidden may now become "allowed" for the small components (that is, the light particle wave functions may be treated as constants over the nucleus). It is convenient to distinguish those components which are small in the spin variable of the transition particle, and those which are small in other variables. These two groups of small components will, in general, also have different selection rules. The result of comparing the selection rules for the three groups of components of the wave functions (the large components, and the two groups of small components), and of discussing the corresponding forms of the electron spectra, show that there may be elements on the second Sargent curve with either

(1) The "allowed distribution form" given by Fermi,
or possibly for light elements (nuclear charge $\leqslant 20$).

(2) Electron distributions which differ from (1), and of types previously discussed (Hoyle 1937; p. 290, fig. 4, I, II),
and that for elements on the third Sargent curve we have the possibilities

(1) The "allowed distribution form" given by Fermi.

(2) Effectively the distribution (1), but with a slight humping at the upper and lower energy limits.

(3) Distributions of the types previously discussed (Hoyle 1937; fig. 4, I, II).

(4) And for light elements (nuclear charge $\leqslant 20$) distributions which differ more widely from the "allowed form" than (3), the shapes of these distributions being similar to (3), but of a more exaggerated form.

On the nuclear forces and the magnetic moments of the neutron and the proton.

By H. Fröhlich, W. Heitler and N. Kemmer. (*Communicated by N. F. Mott, F.R.S.—Received* 1 *February* 1938.)

An attempt is made to explain the properties of the nuclear particles proton and neutron by the hypothesis that these particles are capable of emitting a positive or negative "heavy electron" respectively with a rest mass m_0 between that of the proton and the electron. The existence of those particles has been made probable by cosmic ray observations.

The heavy electrons are assumed to have no (or integral) spin and satisfy Bose-statistics. The wave functions of these particles are assumed to be of vectorial character, the components of which satisfy the Klein-Gordon equation. They are quantised according to the scheme given by Pauli and Weisskopf. Thus, a free heavy electron can exist in three different states of polarization, there are two transverse and one longitudinal wave with given momentum. The interaction with the nuclear particles is found by relativistic arguments and contains—apart from the mass m_0—two arbitrary constants g and f, both with dimensions of an electric charge.

With this scheme we have calculated:

1. The neutron-proton force. It is an exchange force and has a range $1/\lambda = \hbar/m_0 c$. In the ^{3}S-state the force is always attractive. (This would not be the case for a scalar wave function.) g and f can be chosen so that the ^{3}S- and ^{1}S-states have the right position. The experiments suggest $g \fallingdotseq f \fallingdotseq 5$ electron charges.

2. The magnetic moments μ_P and μ_N of the proton and neutron. They are found to be of nuclear dimensions and have the right sign.

3. The mass m_0 can be determined independently from the range of the neutron-proton force and from the magnetic moments. In both ways we find $m_0 \fallingdotseq 100$ electron masses.

4. The proton-proton force is obtained only in the fourth order of approximation and leads to a strong repulsion for distances less than $1/2\lambda$. Attraction and equality with the neutron-proton force could be attained by introducing also neutral particles with mass m_0.

5. The theory leads to a diverging self energy of the proton and neutron.

Photoelectric measurements of the seasonal variations in daylight around 0·41μ from 1930 to 1937. By W. R. G. Atkins, F.R.S. (*Received* 1 *February* 1938.)

In collaboration with H. H. Poole photoelectric cells were standardized and records obtained with a vacuum sodium cell and Cambridge "thread recorder" for 1930 (*Philos. Trans.* 1935, **235**, 1–27; 1936, **235**, 245–72). Further measurements are now summarized and compared with those for 1930, after establishing the constancy of the cell used. The greatest, least and mean values of the daily maximum, in kilolux, are given for each month, also corresponding values for the total vertical illumination in kilolux hours. For the sake of uniformity all the daily curves were measured by the author.

Five years were very similar and averaged 309 kilolux hours a day, but 1934 gave 350 and 1930 gave 414. The greatest sunshine average was 5·17 hr. in 1933. A comparison with the meteorological returns failed to explain satisfactorily the high values for 1930, though radiation at South Kensington was rather high that year. It is possible that specially clear upper air was prevalent in 1930, or it may be that the explanation lies in a variation in the ratio of ultra-violet to green in the solar spectrum, as suggested by Pettit but since contested by Bernheimer.

The coagulation of plasma by trypsin. By J. Mellanby, F.R.S. and C. L. G. Pratt. (*Received* 2 *February* 1938.)

Trypsin digests the fibrinogen contained in oxalated plasma when the amount added is greater than the quantity of antitrypsin in the plasma. Stable, non-oxalated, fowl plasma is subject to the same effect, but certain concentrations of trypsin bring about coagulation. This coagulant action is due to the liberation by trypsin of thrombokinase previously masked in the plasma. Contrary to certain recent claims, trypsin does not activate prothrombase. It is possible to prepare fowl plasma, free from thrombokinase, such that the addition of thrombokinase brings about coagulation whereas the addition of trypsin does not. The results indicate that thrombase is not identical with trypsin and that thrombokinase and calcium cannot be replaced by trypsin in the activation of prothrombase.

Studies on the hypophysectomized ferret. X. Growth and skeletal development. By A. S. Parkes, F.R.S. and I. W. Rowlands. (*Received* 4 *February* 1938.)

Six immature male and two immature female ferrets were hypophysectomized at a body weight of 500–600 g. (7–10 weeks of age). Subsequent body growth in ferrets of both sexes was retarded; in the female the stasis was almost complete but the males continued to grow until the age when the growth in the normal ferret is complete.

Skeletal development was studied by means of X-ray photographs and prepared skeletons. It was found that skeletal growth had been arrested; the bones had a low calcium content and open epiphyses. Stasis in the development of the skull was most marked, this being shown by the absence of sagittal and nuchal crests, open sutures and the retention of all the characteristics of the skull of an immature animal.

The reaction between oxygen and nitric oxide. By E. M. Stoddart. (*Communicated by F. G. Donnan, F.R.S.—Received* 4 *February* 1938.)

It has been shown that the observations of Baker on the non-interaction between intensively dried oxygen and nitric oxide can be repeated, provided that care is taken to ensure that mixing takes place entirely in the oxygen-containing bulb. The only explanation of this phenomenon is that the reaction between the gases is a heterogeneous one, an explanation which fits the observations of Hasche, who showed that the rate of this reaction was lowered by as much as 20% in paraffin coated vessels. The present author suggests that the drying has no effect except perhaps that of removing the adsorbed water film from the glass vessels, thus allowing the surfaces to adsorb a complete gas film in its place. When this gas film is oxygen, no reaction occurs in the mixed gases, but when the gas film is nitric oxide, reaction is possible. At least this explanation agrees with the known facts regarding heterogeneous reactions.

With reference to Bodenstein's observation that the reaction has a negative temperature coefficient, it was seen that he accounted for this fact by assuming a temporary association of nitric oxide molecules in the form of a complex, the life of which diminishes with rise of temperature. The present work indicates that this complex is best formed when the nitric oxide molecules are held close together by adsorption on glass surfaces. If these complex molecules are free to evaporate from the surface and possess a short life in the gas phase, then it is clear that the reaction may appear to be homogeneous when subjected to measurements of its kinetics and that it will have a negative temperature coefficient owing to the shortening of the life of the complex with rising temperature. It is seen from the present experiments that these complex molecules are not formed in the gas phase (as was believed by Bodenstein).

The experiments described as "cleaning" experiments showed that the author's observations were obtainable without any recourse to intensive drying. It was also shown that the admission of water to the non-reactive gases had no effect and therefore inhibition of reaction is wholly due to surface conditions of the containing vessels, "drying" in itself having no effect.

The experimental observations of J. W. Smith were shown to be quite correct but his explanation of his observations needs much modification. A new compound has been discovered which, as far as the author can determine, appears to be $2NO.P_2O_5$. The existence of the nitrogen peroxide-phosphoric anhydride compound described by Smith is unconfirmed. It has been shown that Smith's work does not prove that intensely dried nitric oxide and oxygen are incapable of reaction.

The electrical conductivity of thin metallic films. 3. Alkali films with the properties of a normal metal.

By A. C. B. Lovell. (*Communicated by A. M. Tyndall, F.R.S.—Received* 4 *February* 1938.)

The technique previously described for measuring the electrical conductivity of thin films deposited by evaporation, in very high vacua, on clean substrates, has been modified to include the deposition of thick films of the alkali metals.

Caesium films 10,000 A thick have resistivities only 4 % greater than the bulk metal, and identical temperature coefficients. Thick rubidium and potassium films have rather higher resistivities, but possess the same temperature coefficients as the bulk metal.

Evidence is produced that the films are polycrystalline, and that the higher resistivities are due to a simple residual resistance. The gradation in properties of the thick films is shown to follow consistently from the properties of the very thin films investigated previously.

From these results and other considerations it is concluded that there are no grounds for the belief that thin films differ essentially in structure from the normal metal.

The Zeeman and Paschen-Back effects in strong magnetic fields.

By P. L. Kapitza, F.R.S., P. G. Strelkov and E. I. Laurman. (*Received* 7 *February* 1938.)

1. A method is described for studying the Zeeman and Paschen-Back effects in magnetic fields up to 320,000 gauss.
2. It is shown that the Zeeman splitting is within the limits of experimental error proportional to the magnetic field and obeys the theoretical predictions previously verified only in weaker fields.
3. In strong magnetic fields we were unable to discover any displacement of the centre of gravity of the splitting pattern, which again is in agreement with the theoretical prediction.
4. The Paschen-Back effect was studied in fields up to 300,000 gauss on the beryllium doublet, and it was shown that the splitting accurately followed the theoretical predictions, and that the intensities of the various components agreed qualitatively with the theory.
5. The initial stages of the Paschen-Back effect were also observed for the zinc triplet ^3P–^3S.

The lift and moment on a flat plate in a stream of finite width. By T. H. Havelock, F.R.S. (*Received* 8 *February* 1938.)

The paper gives a new treatment of the problem of a flat plate in a stream bounded by plane parallel walls, including circulation round the plate. The plate is considered as the limiting case of the elliptic cylinder; an integral equation is obtained whose solution by continued approximation leads to expansions for the lift and moment on the plate. The solution is modified to give similar results when the stream is bounded by parallel free surfaces, taking the boundary condition at a free surface in an approximate form; and a further modification gives the case when one boundary of the stream is a plane wall and the other is a free surface. The problem of the elliptic cylinder in general is also considered with reference to the moment of the forces when the stream is bounded by plane walls and when there is no circulation.

Quantum theory of Einstein-Bose particles and nuclear interaction. By N. Kemmer. (*Communicated by S. Chapman, F.R.S.—Received* 9 *February* 1938.)

It is shown that there are four inequivalent but equally simple possibilities of formulating a field theory of Einstein-Bose particles, in which a positive expression for the energy density exists. Any of these formalisms might tentatively be accepted as a description of the "heavy electron". Considerations of relativistic invariance show that two independent expressions for the interaction of these particles with protons and neutrons can be chosen in each of the four cases. Taking account of the interaction terms, the general Hamiltonian form of the theories is stated and the quantization is performed. The resulting proton-neutron potential is determined and it is found that its sign and spin-dependence agrees with reality in only one of the four cases, namely in the case based on the equations of Proca (1936). The (divergent) self energies of the proton or neutron resulting from the interaction studied are evaluated.

The photosensitivity of diphenylamine-*p*-diazonium sulphate measured by the method of photometric curves. By C. F. Goodeve and L. J. Wood. (*Communicated by C. K. Ingold, F.R.S.—Received* 12 *February* 1938.)

The method of photometric curves, used previously to measure the photosensitivity of visual purple solutions, has been applied to the bleaching of diphenylamine-*p*-diazonium sulphate.

The diazonium salt was bleached with light of wave-length 365 mμ. and it was found to have a quantum efficiency of 0·34 ± 0·02, independent of concentration and temperature. The photosensitivity was also found to be unaffected by the removal of dissolved oxygen and by the addition of an internal filter.

These results have been compared with those obtained with visual purple, and their photochemical significance discussed.

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18 MARCH 1938

The control of beating and of micro-fibrillation by means of potassium, calcium and sodium in the chick embryo heart. Potassium fibrillation.

By P. D. F. MURRAY. (*Communicated by Sir Henry Dale, F.R.S.—Received 7 February* 1938.)

A previous paper showed that fibrillation induced by calcium in the medium depends upon a rise in the ratio calcium at the cell surfaces/potassium in the cell interiors. In the present paper experiments similar in character to those described in the first paper show that fibrillation by potassium in the medium is (1) more readily produced in hearts previously rendered potassium-poor than in hearts which are not potassium-poor, (2) probably more readily produced in hearts previously rendered sodium-poor than in hearts which are not sodium-poor. It is concluded that fibrillation by potassium in the medium occurs (1) when the ratio potassium at the cell surfaces/potassium in the cell interiors is abnormally high, (2) probably also when the ratio potassium at the cell surfaces/sodium in the cell interiors is abnormally high.

The energy loss of penetrating cosmic ray particles in copper.

By J. G. WILSON. (*Communicated by P. M. S. Blackett, F.R.S.—Received 10 February* 1938.)

A description is given of measurements of the energy loss of cosmic ray particles in a copper plate, made by means of the cloud chamber method, and the measurements are compared with those previously made in lead.

The results show that the initial reduction of relative energy loss, $(1/E)\,(dE/dx)$, with increasing energy is probably rather less rapid in copper than in lead, but there

is no indication that the Bethe-Heitler value for the radiation loss applies to markedly higher energies in the lighter element. For both lead and copper there is a maximum of relative energy loss at $E \sim 1{\cdot}5 \times 10^8$ e-volts. It is shown that this loss is not accompanied by the secondary particles to be expected if it were due to the emission of collision radiation. Further, the value of the relative cross-section in this region varies with the absorber less rapidly than Z^2, and the position of the maximum is almost independent of Z.

Absorption of this type will become of particular importance for elements of low atomic number, and it is suggested that the absorption with a maximum at $E \sim 1{\cdot}5 \times 10^8$ e-volts is responsible for the anomaly in the sea-level energy spectrum of cosmic rays at $E \sim 2{\cdot}5 \times 10^9$ e-volts.

The structure of the walls of parenchyma in *Avena coleoptiles*. By R. D. Preston. (*Communicated by W. Stiles, F.R.S.—Received* 14 *February* 1938.)

Consideration of both intact cells and of single walls in oat coleoptiles shows clearly that the cellulose chains of the wall are inclined to the transverse plane, in contradistinction to previously accepted ideas. The chains may, therefore, be represented as forming a series of spirals round the cell; and the spiral may be right- or left-handed in different cells, though it retains the same sign throughout in any individual parenchyma. Growth of the coleoptile under constant light conditions, at a temperature of 25° C., appears to involve a change in the inclination of this spiral, in accordance with changes in cell dimension, which may be interpreted in terms of the geometry of the spiral. The ratio of the length of the parenchyma to its girth is shown to be of importance in this respect. If this ratio increases, the spiral becomes steeper; if it decreases, the spiral may be expected to become flatter. It is probable that both turgor forces and active growth of the wall, as well as external conditions, play a part in this change in micellar inclination. Further investigation of coleoptiles, grown under widely different conditions, is clearly necessary before the precise value of such factors can be appreciated.

Some experimental observations for longitude, made by theodolite, fitted with a shutter eyepiece. By J. de Graaff Hunter, F.R.S. (*Received* 14 *February* 1938.)

Precise astronomical position determination is required in geodesy and geophysics. Longitude observations depend on timing star transits, and the observation errors, both systematic and accidental, are relatively large. At fixed observatories this is mostly overcome by the moving-wire micrometer; but the heavy outfit precludes its wide use in field work, where longitude precision is ordinarily much lower than latitude precision.

The present paper describes a new method, applicable to a theodolite, to enable the triangulator to fix both latitude and longitude with equally high precision; with little additional equipment. The basic principle is to observe the position of a star *at instants controlled by the chronometer*; instead of observing the time at which it reaches definite positions.

A special eyepiece contains a shutter, operated electro-magnetically every third second by the chronometer; operation period being say 0·07 sec. Operation may also be every second, for comparison of time with rhythmic radio signals. Further, a scale with horizontal lines is provided in the eyepiece and rendered luminous; while the shutter is not visible. The scale readings of star position at some twenty successive appearances are recorded. Their mean, converted to arc seconds and applied to the corrected vertical reading, gives the zenith distance at a known precise chronometer instant. With suitably chosen stars, both latitude and longitude are determinate.

Observations over several years, made on the shutter principle with a meridian transit, have shown no personality. Recently, with a Tavistock theodolite of 10 in. focus, the M.S.E. of longitude from a pair of stars = ±0·075 sec. ≡ ±0·7 equatorial sec. of arc; the over-all time of observation of the pair being 8 min. This result is fully as precise and rapid as that of a Talcott latitude pair with a zenith telescope of greater power.

The maternal effects on growth and conformation in Shire horse-Shetland pony crosses. By A. WALTON and J. HAMMOND, F.R.S.

(*Received* 15 *February* 1938.)

1. Reciprocal crosses between the large Shire horse and the small Shetland pony have been made by means of artificial insemination.

2. At birth the foals were approximately proportional in weight to the weights of their mothers and about equal to foals of the pure breeds to which the mothers belonged. The cross-foals from the Shire mare were three times the size of the cross-foals from the Shetland mares. Maternal regulation of foetal growth was very marked and obscured any genetic differences.

3. After weaning, when the foals were under the same nutritive conditions, genetic differences appeared. The foals from the Shire mares grew much less rapidly than pure Shire foals, and the foals from the Shetland mares grew much more rapidly than pure Shetlands. At about 18 months an equilibrium point was reached at which the relative growth rates of the cross-foals and the pure Shetland remained constant. At 3 years the difference between the reciprocal crosses is still marked and is apparently permanent.

4. Differences in the proportions of the animals, when size differences are eliminated, were not so marked as differences in weight and the influence of nutrition not so obvious.

5. The mechanisms, by which maternal regulation may be brought about, are discussed and three possibilities suggested: (*a*) maternal regulation of foetal nutrition, (*b*) maternal hormonal control, (*c*) cytoplasmic inheritance.

6. The bearing of these results on the theoretical concept of growth is discussed.

7. The experiments illustrate the interplay of nutritional and genetical factors which are involved in development.

The biological characters of spontaneous tumours of the mouse, with special reference to rate of growth. By A. Haddon. (*Communicated by E. L. Kennaway, F.R.S.—Received* 15 *February* 1938.)

The following findings were obtained in a study based on 336 spontaneous tumours in the mouse. The occurrence of multiple primary neoplasms approached a chance distribution, although the approximation was statistically inadequate to justify the assumption of actual random incidence. In the great majority of cases the linear measurement of tumour size increased linearly with time, although a small proportion showed exponential increase. No significant association could be established between rate of growth and the incidence of metastasis. A significant correlation was found between tumour size attained and the incidence of metastasis. Time of duration of the primary tumour was found to be a highly important single factor in determining the occurrence of metastasis. No significance could be attributed to a somewhat increased proportion of metastasis in mice with multiple primary tumours. Tumours situated in the caudal half of the body were observed to possess a mean growth rate significantly higher than the mean for similar tumours cephalic in position. No relation was found to exist between location of tumour and production of metastases. When tumours of extreme types were studied, it appeared justifiable to conclude that those of differentiated adenomatous structure more often possessed low rates of growth as compared with the higher rates frequently manifested by dedifferentiated and anaplastic histological types. In the case of pregnant animals no evidence was found to suggest that gestation influenced the rate of tumour growth, but parturition and the onset of lactation were not uncommonly followed by a temporary retardation. Slow growth was frequently observed in the earliest stages of tumour development, such examples attaining their maximal growth rate only after a variable and often considerable interval. Mention is made of the possible etiological significance of this phenomenon. Apart from the effect described as consequent on parturition and lactation, retardation during the later stages of growth was mainly due to incidental factors such as bacterial infection of the tumour substance.

The influence of carcinogenic compounds and related substances on the rate of growth of spontaneous tumours of the mouse. By A. Haddon. (*Communicated by E. L. Kennaway, F.R.S.—Received* 15 *February* 1938.)

Parenteral administration of 1 : 2 : 5 : 6-dibenzanthracene in mice bearing spontaneous neoplasms (mainly carcinomata of the mammary gland) resulted in most cases in a prolonged inhibition of the rate of tumour growth. The degree of individual response varied from slight to marked retardation, and a few cases manifested active regression, either partial or complete. The same result, with a corresponding degree of variation, was produced by the carcinogenic substances 1 : 2 : 5 : 6-dibenzacridine, methyl cholanthrene and styryl 430. The non-carcinogenic compounds pyrene and 1 : 2 : 3 : 4-dibenzanthracene provoked either no response or a transient interference with growth rate followed by complete recovery. Instances are given in which the non-carcinogenic substances acenaphthanthracene and 1 : 2 : 5 : 6-dibenzphenazine led to a retardation of growth not different from that produced by carcinogenic compounds. Administration of large doses of oestrone benzoate produced in some cases no inhibition and in others a moderate retardation with a tendency to recovery. The inhibitory response brought about by carcinogenic compounds and by certain related non-carcinogenic compounds is attributed to the possession by such substances of toxicity of a special kind.

The scattering of fast β-particles by mercury nuclei. By F. C. CHAMPION and A. BARBER. (*Communicated by C. D. Ellis, F.R.S.—Received* 17 *February* 1938.)

The scattering of β-particles of about 1 mV energy by mercury nuclei is examined with an expansion chamber for angles of scattering greater than 20°. The absolute number scattered is much less than would be expected from Mott's formula. There is also indication of more inelastic collisions than are predicted by the theory of Bethe and Heitler. Photographs are reproduced which show the sudden stoppage of a fast β-particle with the simultaneous production of what appear to be low energy pairs of positrons and electrons. It is suggested that the results may be qualitatively explained on the assumption of a repulsive field between electrons and nuclei for close distances of approach.

A high-temperature Debye-Scherrer camera and its application to the study of the lattices pacing of silver. By W. HUME-ROTHERY, F.R.S., and P. W. REYNOLDS. (*Received* 17 *February* 1938.)

A high-temperature Debye-Scherrer camera is described for use at temperatures up to 1000° C. The camera is designed primarily for the accurate determination of lattice spacings of metals and alloys. The specimen is contained in a very thin-walled sealed silica tube in order to prevent change of composition of the specimen owing to volatilization, oxidation, etc. The temperature is measured by means of a thermocouple, and can be controlled to within $\pm 1^\circ$ C. of the desired value.

The lattice spacing of silver is determined accurately between 20 and 943° C. The values agree to within 0·0001 A with the data of Scheel for the expansion of massive bars of silver up to 500° C. The values of Keesom and Jansen for the coefficient of expansion of silver in the range -253° C. enable the lattice spacings at low temperatures to be calculated, so that the lattice spacing of silver is now known from 20° above the absolute zero to 18° below the melting-point. It has been found possible to express the results in the form of an equation such that only four constants are required to express the coefficient of expansion over the whole range. This equation is of such a nature that the coefficient of expansion vanishes at the absolute zero in agreement with theoretical requirements. One term in the equation becomes vanishingly small above 0° C., and the coefficient of expansion from room temperature to the melting-point can be expressed in terms of two constants only. The greatest difference between the calculated and observed values is equivalent to 1 part in 10,000 in the lattice spacing. An equation of the same type has been found to hold for other substances.

The dispersion formula for nuclear reactions. By P. L. Kapur and R. Peierls. (*Communicated by R. H. Fowler, F.R.S.—Received* 17 *February* 1938.)

The dispersion formula of Bethe and Placzek, which expresses the cross-section for nuclear reactions in terms of the virtual levels of the compound nucleus and their widths, is derived without using the assumption that the motion of the incident particle within the nucleus can in any approximation be regarded as a one-body problem.

It is possible to derive a formula of the type of the dispersion formula without any assumption at all about the mechanism of the reaction. However, in the general case this formula contains more parameters than the usual dispersion formula and is therefore of less practical value.

The dispersion formula of Bethe and Placzek is shown to hold rigorously if only the widths of all virtual levels which contribute essentially to the expression are much smaller than the distances between the levels.

An X-ray study of the iron-nickel-aluminium ternary equilibrium compound. By A. J. Bradley and A. Taylor. (*Communicated by W. L. Bragg, F.R.S.—Received* 18 *February* 1938.)

X-ray powder photographs of slowly cooled alloys of iron, nickel and aluminium have been used to construct a phase diagram for all compositions. The system falls into two distinct portions. Up to 50 atomic per cent of aluminium there are only body-centred cubic and face-centred cubic structures. Beyond 50 per cent of aluminium, the diagram is extremely complex and will be described in a later paper.

The face-centred cubic phase field includes superlattice structures related to Ni_3Al or Ni_3Fe (α_1), as well as disordered structures (α). It encloses a miscibility gap where two face-centred cubic superlattice phases (α_1 and α_1'') are in equilibrium. As the iron content increases, the gap closes.

The body-centred cubic phase field includes superlattice structures related to Fe_3Al (β_1) and FeAl or NiAl (β_2). There is a two-phase field where the iron-rich β phase (without a superlattice) is in equilibrium with the β_2 phase. In consequence, the system is much more complicated than had previously been supposed. Instead of a two-phase field separating the face-centred cubic and body-centred cubic areas, there are four separate areas, comprising three two-phase fields and a three-phase field. In the latter a face-centred cubic structure (α) is in equilibrium with two body-centred cubic structures (β and β_2). The two-phase fields are $\alpha+\beta$, $\alpha+\beta_2$ and $\beta+\beta_2$ respectively. The corners of the three-phase triangle are placed at the following compositions, α, 62, 31, 7; β, 90, 7, 3; β_2, 28, 41, 31. (The numbers represent atomic per cent iron, nickel and aluminium respectively.)

A note on Guggenheim's theory of strictly-regular binary liquid mixtures.

By G. S. Rushbrooke. *(Communicated by R. H. Fowler, F.R.S.—Received 18 February* 1938.)

An attempt is made to improve and extend Guggenheim's tentative theory of strictly regular binary liquid mixtures by using Bethe's indirect method to avoid the unknown combinatory factor in the partition function for the liquid phase. The expression found in this way for the configurational energy of the mixture differs somewhat from that proposed by Guggenheim, and consequently formulae for the thermodynamic functions are also modified.

The conditions under which such solutions will exhibit critical mixing phenomena are also discussed and the variation with temperature of the concentration at which the liquid mixture begins to separate into two phases is investigated.

Though for simplicity the theory is developed on the supposition that the molecules of the liquid are not close-packed, numerical calculations for face-centred cubic packing, included in the last section, suggest that this does not significantly affect the results.

Investigations on Mediterranean Kala-azar. XI. A study of leishmaniasis in Canea (Crete).

By S. Adler, O. Theodor and G. Witenberg. *(Communicated by Sir Henry Dale, F.R.S.—Received* 22 *February* 1938.)

The epidemiology of leishmaniasis in Canea is discussed. Human visceral leishmaniasis, canine visceral leishmaniasis, and *Phlebotomus major* have an identical distribution in Canea. *P. major* is the only sandfly in Canea of importance for the transmission of visceral leishmaniasis. *P. perniciosus* var. *tobbi* and *P. perfiliewi* are absent, and *P. chinensis* var. *simici* is not common.

The bionomics of *P. major* in Canea are discussed.

The hitherto unknown male of *P. laroussei* is described and the relation of this species to *P. vesuvianus* and *P. canaaniticus* is discussed. *P. vesuvianus* is considered a synonym and *P. canaaniticus* a variety of *P. laroussei.*

The clinical condition of naturally infected dogs is improved and the infection rate in *P. major* fed on these animals reduced by placing them on a diet of fresh meat without any further treatment.

A number of naturally infected dogs have difficulty in barking owing to infiltration of the vocal chords with macrophages and plasma cells.

P. major, *P. chinensis* var. *simici* *P. sergenti* and *P. papatasii* were infected with *Leishmania infantum* by feeding on naturally infected dogs, on Syrian hamsters and a spermophil. The infection rates in *P. sergenti* and *P. papatasii* were very low as compared with *P. major.*

P. sergenti, *P. papatasii* and *P. major* were infected with *L. tropica* by feeding on lesions in human beings in Canea. The infection rate in *P. papatasii* is very low as compared with that in *P. sergenti*. *P. papatasii* plays no significant role in the transmission of *L. tropica* in Canea, and *P. sergenti* appears to be the main vector of *L. tropica* in Canea.

The Canea strain of *L. tropica* is biologically different from the Palestinian one. In contrast to the latter it produces a very low infection rate in *P. papatasii* both in Greece and Palestine.

A human being was infected with *L. tropica* by the inoculation of flagellates from an artificially infected *P. sergenti*.

The general distribution of sandflies of the major group with relation to visceral leishmaniasis in the Old World is discussed.

The accurate analysis of gaseous mixtures.

By B. LAMBERT and D. J. BORGARS. (*Communicated by C. N. Hinshelwood, F.R.S.—Received 22 February* 1938.)

An apparatus is described for the accurate analysis of small volumes of gas mixtures.

It has been found possible to obtain results accurate to 0·02 per cent for the determination of the percentage content of one constituent of a gaseous mixture using about 10 c.c. of gas. The apparatus is also suitable for "microanalyses" of gaseous mixtures using 1 to 2 c.c. of gas mixture and results accurate to 0·1 per cent are obtainable.

Difficulties associated with the accurate determination of carbon monoxide in a gaseous mixture are discussed and methods of overcoming them are described.

Atomic rearrangement process in the copper-gold alloy Cu_3Au. II.

By F. W. JONES and C. SYKES. (*Communicated by W. L. Bragg, F.R.S.—Received 24 February* 1938.)

The relation between the size of nuclei and electrical resistance has been determined for the copper-gold alloy Cu_3Au in both the annealed and cold worked states. The experimental results are interpreted using the hypothesis that nuclei are antiphase with narrow boundaries. For large nuclei a linear relation, between resistance and the number of boundaries per unit length, is found from which the reflexion coefficient of a single boundary has been deduced. This reflexion coefficient is the same for both cold worked and annealed material although the rates of growth of the nuclei in the two cases differ considerably.

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5 APRIL 1938

On the theory of heavy electrons and nuclear forces. By H. J. BHABHA. (*Communicated by R. H. Fowler, F.R.S.—Received* 28 *February* 1938.)

A theory is developed based on the idea that the proton and neutron are two states of the same particle, which can go over from one state to the other by the emission of a charged particle of mass intermediate between those of the proton and electron, as originally suggested by Yukawa. These "U-particles" are described by four wave functions. Quantization of the theory leads as usual to positive and negative U-particles with a spin of one unit. The U-particles are identified with the heavy electrons of cosmic radiation. The theory leads uniquely to short range forces of the Heisenberg and Majorana type of such a sort as to allow one to make the ground state of the deuteron the triplet state, *the sign of the Majorana force being not at our choice*. The range of the forces is connected with the mass of the U-particles as before, and demands a mass of some two hundred times the electron mass. The relativistic generalizations of a pure Heisenberg and pure Majorana force are given. The relativistic scattering of U-particles by protons and neutrons is calculated. The theory also leads to showers of Heisenberg's type, but consisting of heavy electrons and heavy particles only.

The statistical mechanics of condensing systems. By M. BORN and K. FUCHS. (*Communicated by E. T. Whittaker, F.R.S.—Received* 1 *March* 1938.)

J. E. Mayer's general theory of gases was the subject of a discussion at the Van der Waals Centenary conference in Amsterdam (1937) where objections were raised against his explanation of condensation. We present a new form of Mayer's theory using the method of complex integration by which it is possible to show rigorously that Mayer's statements are essentially correct.

The starch-iodine coloration as an index of differential degradation by the amylases.

By C. S. Hanes and M. Cattle. (*Communicated by F. F. Blackman, F.R.S.—Received* 7 *March* 1938.)

The alterations in iodine coloration have been followed by a spectrophotometric method during the action of five representative amylases on starch. It is found that the so-called saccharogenic and dextrinogenic types of amylase are distinguishable from the early stages of their action by the manner in which the absorption characteristics of the iodine compounds are changed. The relation between the alterations in the iodine colouring property and the liberation of reducing groups throughout the different degradative processes has been examined; such *colour/reducing power* relationships are shown to constitute a diagnostic criterion for comparing the action of different amylases.

The problem of the constitutional basis of the iodine colouring property of starch and starch dextrins is considered in the light of these quantitative data. It would appear that the hue of iodine colour exhibited by a particular product is to a large extent independent of the length of its chain molecule but is governed mainly by some factor such as the mode or degree of association of the chemical units as they exist in molecular aggregations. This hypothesis is in harmony with the available evidence from purely chemical studies, on the one hand, and with conceptions of the mode of attack of the starch molecule by the different enzymes, on the other.

The dielectric polarization of a *n*-long chain ketone at constant volume and variable temperature.

By A. Müller. (*Communicated by Sir William Bragg, P.R.S.—Received* 7 *March* 1938.)

The present work is a continuation of a previous investigation on the dielectric polarization of a long chain ketone. The substance, instead of being allowed to expand at atmospheric pressure, is now kept at constant volume. This constraint produces a very marked change in the behaviour of the substance when compared with previous results. It is shown that the rise in polarization at constant pressure goes parallel with the large expansion which precedes the melting.

With regard to the theory of cooperative phenomena the present experiments show the effect of a variation of the boundary conditions upon the behaviour of the substance.

Transference of induced food habit from parent to offspring, III.

By D. E. Sladden and H. R. Hewer. (*Communicated by E. W. MacBride, F.R.S.—Received* 10 *March* 1938.)

A modification of food habit has been induced in stick insects and has been transmitted through a number of parthenogenetic generations. The anomalies of the earlier communications have been satisfactorily explained.

Formation of negative ions at metal surfaces. By R. A. Smith. (*Communicated by N. F. Mott, F.R.S.—Received* 10 *March* 1938.)

Various processes of formation of negative ions at metal surfaces, and the conditions under which they may take place are discussed. Detailed calculations are given for the conversion of Hg^+ ions into Hg ions at a nickel surface. The positive ions are assumed to be neutralized by capturing an electron from the metal, and to form excited atoms which subsequently capture another electron in falling into their ground state. This process is shown to account for the experimental results of Arnot and Milligan. The calculated probability of formation by this process is $1{\cdot}4 \times 10^{-3}$ for 200 V Hg^+ ions striking a nickel surface normally. The process of simultaneous capture of two electrons from the metal is shown to be improbable.

A negative ion formed near a metal surface for which the work function is greater than the electron affinity of the corresponding atom or molecule will have a considerable chance of being neutralized through one of its electrons passing into an unoccupied level in the metal. It follows that slow positive ions or metastable atoms will be ineffective as a source of negative ions.

The formation of atomic negative ions from molecular positive ions is discussed. Calculations for the formation of H^- ions from H_2^+ ions and from protons are given. The value obtained for the probability of formation of H ions from 140 V protons striking a surface with work function less than 3·37 e-volts is about 4×10^{-3}. It is shown that H^- ions will not be formed from protons at a surface with work function greater than 3·37 e-volts unless simultaneous capture of two electrons takes place. For formation of H^- ions from H_2^+ the observed probability is only $1{\cdot}04 \times 10^{-5}$ for 200 V H_2^+ ions striking a nickel surface. This low value is shown to be due to the small probability of formation of the intermediate excited H atoms by dissociation of the incident ions. Clearly, further experiments, using separated beams of protons and H_2^+ ions, are required in order to clarify the processes taking place when atomic negative ions are formed from molecular positive ions.

Showers produced by the penetrating cosmic radiation. By W. Heitler. (*Communicated by N. F. Mott, F.R.S.—Received* 7 *March* 1938.)

The theory of the heavy electron developed by Fröhlich, Heitler and Kemmer, has been applied to the passage of fast heavy electrons through matter. It is shown that various types of showers are produced (Y = heavy electron, P = proton, N = neutron).

(1) $Y^+ + N = P + \hbar\nu$. The light quantum produces an ordinary cascade shower.

(2) Multiple processes of the type $Y^+ + N = P + Y^+ + Y^-$ and of higher order are possible. They lead to penetrating showers produced by penetrating particles.

(3) In a heavy nucleus: $Y^+ + N = P$. The energy of Y^+ is restored in the nucleus which subsequently evaporates emitting a few protons and neutrons (proton shower) accompanied by electrons and possibly also heavy electrons.

The inverse process to (1) leads to the creation of heavy electrons by light quanta. It is shown that the order of magnitude of the cross-section is sufficient to explain all heavy electrons at sea-level as secondaries produced in the high atmosphere.

Owing to the breakdown of the theory for energies $\gg \mu c^2$ ($\gg 10^8$ e-volts) all these processes can only be discussed qualitatively and their cross-section can only be calculated approximately for energies of the order 10^8 e-volts or less. For this energy the cross-sections have a reasonable order of magnitude.

The formation of helium molecules.

By F. L. ARNOT, Ph.D. and MARJORIE B. M'EWEN, B.Sc. (*Communicated by H. S. Allen, F.R.S.—Received* 11 *March* 1938.)

An investigation of the formation of ionized molecules in argon, neon and helium has been made by the balanced space-charge method which had previously been used by the authors to study the formation of mercury molecules. No evidence of molecular ionization was found in argon and neon. In helium molecular ionization sets in at the resonance potential. The results show that these molecular ions are formed by the attachment of metastable atoms to normal atoms according to the process

$$\mathrm{He}(1s^2,\ {}^1\mathrm{S}_0) + \mathrm{He}'(1s2s,\ {}^3\mathrm{S}) \rightarrow \mathrm{He}_2^+(1s\sigma^2 2p\sigma,\ {}^2\Sigma_u) + \epsilon.$$

The appearance potential of the molecular ions is the energy of the $2\,^3$S state, 19·77 V, which is 1·19 V greater than the ionization potential of the helium molecule.

Attention is drawn to the fact that these molecular ions are formed from excited atoms in S-states, whereas we have shown that mercury molecular ions are apparently formed only by excited atoms in P-states.

An accurate determination of the range-distribution curve of the radioactive alpha particles from ^{8}Li.

By C. L. SMITH and W. Y. CHANG. (*Communicated by J. D. Cockcroft, F.R.S.—Received* 12 *March* 1938.)

The paper describes a method for determining the number-range distribution curve for the α particles from radioactive lithium. These α particles are shown to consist of a continuous distribution extending up to a maximum of $6{\cdot}95 \pm 0{\cdot}1$ cm. ($7{\cdot}75 \pm 0{\cdot}05$ mV). The shortest range observed was 0·65 cm. (1·2 mV) and in the region from 0·65 cm. up to 6·6 cm. there is a linear relation between the logarithm of the number of α particles and their range. Comparison is made with the distribution curves obtained by other workers and it is also shown that the upper limit of the energy of the α particles explains the failure to observe the protons emitted in the reaction:

$$^7\mathrm{Li} + {}^2\mathrm{D} = {}^8\mathrm{Li} + {}^1\mathrm{H}.$$

Upper and lower limits for the mass of ^{8}Li are calculated from the data and shown to be respectively 8·0253 and 8·0246.

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27 April 1938

The structure and relations of the human premaxilla. By E. H. Johnson. (*Communicated by F. Wood Jones, F.R.S.—Received* 10 *March* 1938.)

The structure and relations of the human premaxilla have been described. It has been shown to be vestigial in character compared with the homologous bones in lower animals.

The premaxillae are completely covered on the facial aspect by the incisor processes of the maxillae. "Suture lines" on the facial surface have been shown to be due to superficial fissures between the ridges of developing bone on the frontal process of the maxilla. From the conditions observed in the region of the floor of the nasal cavity, it seems that the double narial margins which occur in prognathous races are attributable to an incomplete passage of the incisor processes of the maxillae towards the anterior nasal spine.

The anterior alveolar walls of the incisor teeth are maxillary.

The ascending process of the premaxilla becomes entirely restricted to the internal nasal surface of the maxilla, because of relative differences in the rate of bone growth in this region. In cleft palate, the variation in position of the cleft is not due to a division through the premaxilla, but to the lateral incisor and its alveolus becoming displaced to the lateral side of the cleft. Pathological "exfoliation of the premaxilla" is not indicative of its structural form.

The premaxilla in man has been compared with that in other Primates and has been shown to differ in form, relations, and the time of closure of the sutures. The peculiar shutting off of the premaxilla on the face by the incisor processes of the maxillae is to be regarded as a specific character of man.

The distribution of carbon dioxide in the hen's egg. By J. Brooks and J. Pace. (*Communicated by Sir Joseph Barcroft, F.R.S.—Received* 14 *March* 1938.)

The combined CO$_2$ of egg-white in equilibrium with different partial pressures of CO$_2$ was measured over the range $p = 0{\cdot}05\text{–}1{\cdot}0$ atm. CO$_2$ at 25° C. An increase in CO$_2$ pressure increased the concentration of combined CO$_2$. It was assumed that the combined CO$_2$ was present in the form of bicarbonate. On this basis the buffer value per g. protein was $4{\cdot}8 \times 10^{-5}$ for the pH range 6·6–7·8. The reasonable values calculated for the pH of the white and the buffer value of the proteins supports the assumption that the amount of carbamino—CO$_2$ present is small.

The value of the Bunsen solubility coefficient for CO$_2$ and white at 25° C. was 0·71. This value is about 8 % greater than the value calculated from the salt and water content of the white. It is suggested that, as in blood serum, the excess solubility is caused by the presence of traces of lipoids.

The uptake of CO$_2$ by shell and yolk was measured. The retention of CO$_2$ and buffer values of egg-white and the relation between the salts of shell and white have been discussed.

Radioactive nodules from Devonshire. By M. Perutz. (*Communicated by J. D. Bernal, F.R.S.—Received* 15 *March* 1938.)

Globular concretions surrounded by bleached haloes are found in Permian Beds west of Budleigh Salterton, Devonshire. Sections show that their internal structure is complex, a sandy matrix being impregnated by a black hard material in radial and concentric sheets. Contact photographs of a plane section show a distribution of radioactive material closely connected with the structure of the black impregnation. From measurements of the intensity of the radioactivity on an ionization counter a content of 0·4 % uranium was calculated and later confirmed by chemical analysis.

The physical properties of the black part are caused by an apparently amorphous impregnation of vanadium oxides, various other ores also being present in smaller concentrations. Three classes of nodules are described and the possibilities of their origin are discussed.

The last part contains a chemical and geometrical description of the bleached haloes, and after discussion of explanations of their formation suggested by earlier authors the ionizing effect of radon is proposed as an agent which might be partly responsible for the bleaching.

Application of reciprocity to nuclei. By M. Born. (*Communicated by E. T. Whittaker, F.R.S.—Received* 15 *March* 1938.)

The formula for the distribution of quantum states, which follows from the principle of reciprocity and the assumption of a closed p-space, is applied to some properties of nuclei. The results can be considered as confirmations of the hypothesis. The mass of a particle moving with velocity of light and maximum momentum is of the same order as that of the particles which Yukawa has introduced.

Classical theory of radiating electrons. By P. A. M. DIRAC, F.R.S. (*Received* 15 *March* 1938.)

The object of the paper is to set up in the classical theory a self-consistent scheme of equations which can be used to calculate all the results that can be obtained from experiment about the interaction of electrons and radiation. The electron is treated as a point charge and the difficulties of the infinite Coulomb energy are avoided by a procedure of direct omission or subtraction of unwanted terms, somewhat similar to what has been used in the theory of the positron. The equations obtained are of the the same form as those already in current use, but in their physical interpretation the finite size of the electron reappears in a new sense, the interior of the electron being a region of space through which signals can be transmitted faster than light.

Self-consistent field with exchange for calcium and argon. By D. R. HARTREE, F.R.S. and W. HARTREE. (*Received* 16 *March* 1938.)

The differences between the atomic wave functions calculated by the method of the self-consistent field with and without exchange can in certain cases be plotted in such a way that the results for different atoms fall on approximately the same curve, which then can be used for interpolating between different atoms.

Wave functions so estimated using the results already calculated for Cl^- and Ca^{++} have been used as the basis of calculations of the self-consistent field for K^+ and Ar. For K^+ all the (nl) wave functions have been calculated; the results showed that for Ar all but the outer [$(3s)$ and $(3p)$] wave functions could be interpolated to adequate accuracy by the method mentioned.

Results are given, and values of the diamagnetic susceptibilities calculated and compared with observed values.

Studies of the post-glacial history of British vegetation. By H. GODWIN and M. H. CLIFFORD. (*Communicated by Sir Albert Seward, For.Sec.R.S.—Received* 16 *March* 1938.)

The plant remains of the fen deposits have been analysed, and stratigraphy has been determined from profiles and from extensive borings: the methods of pollen analysis have been employed to indicate the drift of local vegetation phases on the fens themselves.

Part I deals with the Woodwalton Fen area, a part of the fenland margin south of Peterborough, where the fen peats have been little damaged by drainage and peat cutting. It is shown that on the landward side there is a single peat bed, which is separated into two not far from the fen margin, by the tapering edge of a bed of fen clay shown by foraminiferal and diatom analysis to have been laid down in brackish water. This clay, which must have represented a marine transgression, interrupted a phase of extensive development of fen woods, at first mainly alder-oak, and later pine-birch. Tree remains are very abundant. The peat above the fen clay showed clear evidence of the development of acidic sphagnum peat of the kind found only in raised bogs ("Hochmoore"). This type of peat was previously unrecognized in the fens, and it reflects conditions of freedom from flooding by alkaline water. It is probable that this phase corresponds with the Bronze Age, and that in the succeeding Iron Age conditions changed sharply. The acid sphagnum peat is overlaid by the calcareous lake marl of Ugg Mere and Trundle Mere, lakes which were only recently drained. They are thus shown to have had a very recent origin.

Part II of the paper extends the observations in the Woodwalton area towards the sea and towards the southern half of the fens. A series of long sections has been constructed which converge towards Wisbech (upon the main estuary of the last phase of fen history). These sections show that the upper and lower peats, separated by the fen clay, occur regularly and continuously over the entire area. On the seaward side they are overlaid by an upper layer of semi-marine silt deposited in the Romano-British period. By a long series of shallow bores the above series of sections was tied to the known profiles at Wood Fen, Ely. Thence it was clear that the phases of lower peat, fen clay, upper peat and upper silt could be accepted as broad major divisions across the whole of the southern part of the Fenland.

Peat formation in the Boreal period was restricted to places of local wetness, such as deep river valleys. A dry phase at the Boreal-Atlantic transition corresponded with a late Tardenoisian culture horizon. Peat formation became general in the fens in the Atlantic period, and the fens became wooded in the Neolithic period, during the end of which time, or just after which, there was an extensive but shallow marine transgression which caused the fen clay to be formed. The succeeding period in the fens began with "A" Beaker culture and during the Bronze Age they were dry: the fens were either wooded or formed raised-bogs, and were fairly habitable. The ensuing Iron Age must have been wet. The Roman period was marked by the deposition of considerable thicknesses of semi-marine silt in a wide belt on the seaward side of the fens, and in tongues along the courses of the estuaries. There were human settlements upon the silt whilst it was forming, and its present surface shows the remains of dense occupation. The great meres of the Fenland probably formed either in the Iron Age or the Romano-British period.

On the theory of scattering of light. By HANS MUELLER. (*Communicated by R. H. Fowler, F.R.S.—Received* 16 *March* 1938.)

The Krishnan effect can be explained if the fluctuations of the optical anisotropy in the medium are not independent in neighbouring volume elements. By applying Brillouin's method to longitudinal and transversal waves the Krishnan effect is calculated for arbitrary directions of observation. It is found that the reciprocity relation is always valid. Every substance which has a Krishnan effect should show a Mie effect. Both effects are related to the photoelastic properties in the case of solids, and to the constants of streaming birefringence in the case of liquids.

The Krishnan and Mie effects must occur for temperature scattering in solids. In isotropic solids the scattered light must consist of two pairs of Doppler components, for crystals three different Doppler shifts can be expected. The Krishnan effect can occur in ordinary liquids if Lucas' transversal waves give a noticeable contribution to the scattering. For temperature scattering the depolarization $\rho_h = V_h/H_h$ is always larger than 1.

A new theory of the scattering of light in glasses is proposed. It is based on the assumption that glasses contain a random distribution of "frozen in" strains. Slightly below the solidification temperature these strains are normal ones, but at lower temperatures shearing strains are created due to temperature contraction. ρ_h is smaller than 1 because the shearing strains are always smaller than the normal strains. It is found that Krishnan's data are in excellent agreement with those calculated for glasses for which the photoelastic constants are known.

Liquids with molecular clusters can be treated as if they were uniform liquids with a distribution of internal strains. They show $\rho_h < 1$ because the strains are predominantly normal ones. A relation between the Mie effect for critical opalescence and the deviation from the λ^{-4} law is found to agree with Rousset's observation.

It is pointed out that scattering data furnish a Fourier analysis of the optical variations within the medium and can be used to determine the size of molecular clusters and of colloidal particles.

Gans' theory of scattering by molecular clusters is discussed and it is shown that it agrees with Krishnan's reciprocity relation.

The two-stage auto-ignition by hydrocarbons. By G. P. Kane. (*Communicated by A. C. G. Egerton, F.R.S.—Received* 17 *March* 1938.)

Previous investigations into the spontaneous ignition under pressure of the higher paraffins and olefins containing more than three carbon atoms have shown that in the temperature range between *ca.* 270–400° C., ignition occurs by a two-stage process preceded by an induction lag t_1 before the formation of a cool flame and a second lag t_2 before the subsequent ignition of the cool flame products; increasing pressure shortens both these lags, and although kinetic relationships have been developed it has not been possible adequately to test them owing to the extreme violence of the ignitions at pressures much above the minimum ignition pressure.

An optical recording manometer is described whereby it has been possible to measure t_1 and t_2 at pressures up to 15 atm. with an accuracy of 1/100th of a second.

With propane, t_2 decreases more rapidly than t_1 with increasing initial pressure and at a critical pressure (about 8 atm.) the two-stage is replaced by a single-stage process; the induction lag then decreases very rapidly with pressure with propylene, where the induction lags are much greater, no such transition had occurred at pressures up to 12 atm.

The bearing of these results both on the nature of the kinetic processes operative and on the problem of "knock", is briefly discussed.

The morphology of the brachial plexus, with a note on the pectoral muscle, and the twist of its tendon.

By W. HARRIS. (*Communicated by W. Trotter, F.R.S.—Received* 17 *March* 1938.)

The primary or ventral divisions of the spinal nerves subdivide again into dorsal and ventral nerves to supply the dorsal and ventral muscles and skin of the limb, dorsal joining dorsal and ventral joining ventral divisions.

The simplest type of plexus consists of two cords, dorsal and ventral, formed by the union of the dorsal and ventral subdivisions of all the primary nerves entering the plexus, as in frogs and toads, and in birds. In mammals the original ventral cord, and to a less extent the dorsal cord becomes split up into different nerve trunks, though dorsal and ventral nerves remain apparently strictly apart.

Examination of the reptilian type of plexus in salamanders, turtles, lizards, crocodiles and the Monotremata demonstrates the passage of dorsal fibres into the median and ulnar branches of the ventral cord, probably for convenience of carriage, facts which suggest that essentially dorsal fibres in Man must enter the ventral trunks early, and thus explain the dorsal cutaneous and muscular supply in the hand by the median and ulnar nerves in Man.

The marsupial plexus is a primitive mammalian type, and varies little with the structure of the animal.

Short-necked mammals like the manatee and the Cetacea have five to seven nerves in the plexus, and long-necked forms like the llama and horse have a compressed plexus.

The Carnivora and quadrupedal Ungulata have lost the fifth cervical nerve from the plexus.

The second dorsal nerve may be considered to be a disappearing feature in the mammalian plexus.

In the Primates with arboreal life and increasing use of the deltoid, the fifth cervical nerve reappears and the plexus becomes increasingly prefixed up to the anthropoids, and the pectoral muscle develops a twist of 180° in the tendon of its lower half, aiding the power of climbing.

The nuclear magnetic moment of copper.

By S. TOLANSKY and G. O. FORESTER. (*Communicated by P. M. S. Blackett, F.R.S.—Received* 18 *March* 1938.)

The doublet hyperfine structures of the resonance lines of the copper spectrum, $\lambda\,3247$ and $\lambda\,3274$, have been measured with a quartz Lummer plate. The lines are produced free from reversal effects, the doublet separations being respectively 379 and 405 × 10^{-3} cm.$^{-1}$. The following hyperfine structure interval factors are calculated. $3d^{10}\,4s\,1\,^2S_{\frac{1}{2}} = 197{\cdot}5$, $3d^{10}\,4p\,2\,^2P_{\frac{1}{2}} = 14$ and $3d^{10}\,4p\,2\,^2P_{\frac{3}{2}} = 4{\cdot}8$ (all in cm.$^{-1} \times 10^{-3}$). The mean nuclear magnetic moment for the two copper isotopes, 63 and 65, is derived from the ground state, $3d^{10}\,4s\,1\,^2S_{\frac{1}{2}}$. The value found is $\mu = 2{\cdot}47$ nuclear magnetons, this being probably a better estimate than that given by other terms, since the ground state is spherically symmetrical and thus not affected by quadrupole moment of the nucleus. By adopting Schüler and Schmidt's value for the ratio of the magnetic moments of the two isotopes, it is found that $\mu\,\mathrm{Cu}_{63} = 2{\cdot}43$ and $\mu\,\mathrm{Cu}_{65} = 2{\cdot}54$ nuclear magnetons.

The disintegration of boron by slow neutrons. By C. O'CEALLAIGH and W. T. DAVIES. (*Communicated by E. V. Appleton, F.R.S.—Received* 18 *March* 1938.)

The disintegration of boron by slow neutrons—$_5B^{10} + _0n^1 = _3Li^7 + _2He^4 + Q_1$—has been studied in an expansion chamber. Evidence has been found for the emission of three groups of heavy particles of ranges 4·25 ± 0·2 mm., 7·15 ± 0·25 mm. and 8·9 ± 0·4 mm. in air at 15° C. and 760 mm. The 4·25 mm. group is clearly due to the lithium nucleus. On the basis of the recent range-energy determinations of Blewett and Blewett, the 7·15 and 8·9 mm. groups, which must consist of α-particles, would lead respectively to energy releases of 2·45 and about 3×10^6 e-volts. Substitution in the equation of reaction of the masses at present accepted leads to a value for Q_1 of $2{\cdot}99 \times 10^6$ e-volts. The 8·9 mm. α-particles would correspond, therefore, to the production of the Li^7 nucleus in the ground state, and the 7·15 mm. particles to an excited nucleus having an energy of $0{\cdot}55 \pm 0{\cdot}15 \times 10^6$ e-volts above the ground state.

Evidence based on the emission of two groups of protons in the reaction

$$_3Li^6 + _1D^2 = _3Li^7 + _1H^1$$

leads to the prediction of one low-lying excitation level of Li of $0{\cdot}44 \times 10^6$ e-volts. This is confirmed by absorption measurements on the γ-radiation accompanying this disintegration. Our value $0{\cdot}55 \pm 0{\cdot}15 \times 10^6$ e-volts is in satisfactory agreement with the above figure. A study of the γ-radiation arising from the non-capture excitation of Li^7 by α-particles would seem to indicate the presence of two low-lying excitation levels of 0·6 and $0{\cdot}4 \times 10^6$ e-volts, but it is possible that transitions to the $0{\cdot}6 \times 10^6$ e-volts state are not allowed in the above reactions.

The heteropycnosis of sex-chromosomes and its interpretation in terms of spiral structure. By M. J. D. WHITE. (*Communicated by J. B. S. Haldane, F.R.S.—Received* 22 *March* 1938.)

There appear to be two kinds of heteropycnosis met with in the chromosomes of the *Orthoptera saltatoria*, which may be called the reversible and non-reversible types. In the reversible type the same chromosome may show both positive and negative heteropycnosis at different stages. In the non-reversible type only positive heteropycnosis is seen. During negative heteropycnosis the chromosome does not undergo as much thickening as in the "control" autosome. In positive heteropycnosis the chromosome undergoes more thickening.

Both positively and negatively heteropycnotic chromosomes have a spiral structure at metaphase. The number of gyres in the spiral per unit length is believed to be inversely proportional to the diameter of the chromosome, so that it will be greater in negatively heteropycnotic chromosomes than in positively heteropycnotic ones. The direction of coiling of the spiral is not constant in the *X*-chromosomes of the Tettigonidae, i.e. the same chromosome may show either right-handed or left-handed coiling. In the spermatogonial divisions the direction of coiling may be reversed at the spindle attachment of the chromosome or elsewhere.

A new and anomalous type of meiosis in a mantid. By M. J. D. WHITE. (*Communicated by J. B. S. Haldane, F.R.S.—Received* 22 *March* 1938.)

The meiosis of *Callimantis* takes place in a manner which is entirely unique. No chiasmata are formed and the first meiotic division is consequently reductional for all parts of the chromosomes. The usual diplotene and diakinesis stages are completely absent. Unlike other mantids hitherto studied this species (*C. antillarum* Sauss.) is *XO* in the male.

The problem of *n* bodies in general relativity theory. By Sir ARTHUR EDDINGTON, F.R.S. and G. L. CLARK. (*Received* 22 *March* 1938.)

The motion of a system of two bodies (e.g. a double star) is investigated as far as terms of the second order in the potentials. Contrary to a result obtained by Levi-Civita in 1936, we find no secular acceleration of the centre of mass of the system.

The work revealed an error in the standard formulae for the line-element of a system of *n* bodies, given by de Sitter in 1936. As a result of correcting the error, the equivalent mass *M* of the system is found to be

$$M = E + \tfrac{1}{2}d^2C/dt^2,$$

where *E* is the energy (including potential energy) and *C* the moment of inertia about the centre of mass.

The secretion of crystalloids and protein material by the pancreas. By S. A. KUMAROV, G. O. LANGSTROTH and D. R. MCRAE. (*Communicated by J. S. Foster, F.R.S.—Received* 25 *March* 1938.)

The concentrations of Na, K, Ca, Cl, HCO_3, and protein and non-protein nitrogen, were determined in series of samples of pancreatic juice secreted by dogs in response to (*a*) constant rate of administration of secretin, (*b*) varied rate of administration of secretin, and (*c*) interrupted administration of secretin (rest period, 2 hr.). The use of quantitative spectroscopic methods of analysis in the determination of the metals, and of absorption spectrum methods in the study of the protein composition of the secretion, were important features of the technique.

The fact that the observed concentrations of metals in the secretion are independent of the degree of activity of the gland, is considered to indicate that the glandular membranes offer little resistance to the passage of simple inorganic ions. The *apparent* marked differences in permeability to different ions, as indicated by Ball's (1930) injection experiments, are probably due to a transformation of part of the injected substances to "non-diffusible" forms in the blood stream.

The observed increase in the bicarbonate concentration with increasing rate of secretin administration, and the compensatory relation with the chloride concentration, is interpreted on the basis of (*a*) the formation of at least a part of the bicarbonate as a product of metabolism within the glandular cells, and (*b*) the action of membrane forces, probably of an electrical nature.

A correlation of absorption spectrum measurements with protein nitrogen data admits of two alternative interpretations; (*a*) only one type of absorbing protein is present in the secretion, and a part of all of this serves as a carrier for enzymatically active groups, or (*b*) several types of absorbing protein are present, and these, whether enzymatically active or not, are always secreted in constant proportions.

The interpretation of protein nitrogen and certain other data is given in the accompanying paper.

The processes of synthesis and secretion of protein material in the pancreas.

By S. A. KUMAROV, G. O. LANGSTROTH and D. R. MCRAE. (*Communicated by J. S. Foster, F.R.S.—Received* 25 *March* 1938.)

A theory of the process of synthesis of protein material by the pancreas, and its secretion in response to secretin administration, is developed. The theory leads to expressions which describe quantitatively the behaviour of the protein output in samples of secretion obtained under widely varied experimental conditions. It permits some insight into the fundamental nature of certain of the glandular processes, as well as the calculation of various factors not directly observable in critical experiments. It is rich in suggestions for new problems in connexion with the secretory processes.

The applicability of the Gibbs' adsorption theorem to solutions whose surface tension curves exhibit minima or horizontal portions.

By J. W. MCBAIN, F.R.S. and G. F. MILLS. (*Received* 25 *March* 1938.)

The classical Gibbs' adsorption theorem appears to be a limiting law applying to cases where mutual repulsion or oriented dipoles upon the surface and the effects of submerged double layer and of electrification do not also condition the surface tension.

All aqueous and ionizing systems involve the important factor of a submerged double layer of greater or lesser development, powerfully affecting the surface tension, appreciable even for insoluble oil films on water or for pure water itself.

Abundant examples of "Type 3" surface tension curves have been obtained by many authors by every experimental method. In these, the static or true equilibrium surface tension is very greatly lowered in extreme dilution, thereafter remaining constant or passing through one or more maxima or minima, although the dynamic or immediate surface tension is several times greater, nearly that of water. Application of the classical Gibbs' theorem in its exact form to these cases yields a series of obviously impossible results, no matter in what form they are calculated. The different reasons adduced by many authors for avoiding such applications are seen to be gravely at variance with the foundations of chemical thermodynamics.

The factors of orientation and submerged double layer omitted in the Gibbs' formulation are indicated.

It follows from the Gibbs' equation that the surface of solutions of soluble substances is very deep as compared with molecular dimensions, in contrast to the familiar conception of insoluble films on water.

The first spark spectrum of platinum. By A. G. Shenstone. (*Communicated by R. H. Fowler, F.R.S.—Received 25 March* 1938.)

The paper reports observations on the Spectrum Pt II and classifies a large number of levels. A list of lines is also given including (i) all identified lines between $\lambda 976$ and $\lambda 1242$, (ii) all certain low transition lines and all identified high transition lines between $\lambda 1242$ and $\lambda 4514$.

The adsorption of vapours at plane surfaces of mica. Part II. Heats of adsorption and the structure of multimolecular films. By D. H. Bangham and S. Mosallam. (*Communicated by D. L. Chapman, F.R.S.—Received 25 March* 1938.)

The results are given of measurements of the quantities of benzene, methyl alcohol, and carbon tetrachloride adsorbed at a known surface area of mica at pressures approaching saturation. The adsorption energy is constant whilst the first molecular layer is being formed, but its completion is marked by an abrupt decrease to a value near the normal heat of liquefaction. The graphs of apparent film thickness against relative pressure for the three substances are not widely divergent over much of the range investigated, but the isotherms show discontinuities or sharp changes of curvature at adsorption values which are thought to be related to molecular spacings in the bulk condensed phases. Approximate values are given for the surface tension lowering of the mica, caused by the saturated vapours.

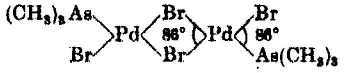
The swelling of charcoal. Part V. The saturation and immersion expansions and the heat of wetting. By D. H. Bangham and R. I. Razouk. (*Communicated by D. L. Chapman, F.R.S.—Received 25 March* 1938.)

The percentage linear expansion x_s of a rod of inactive wood charcoal when exposed to the exactly saturated vapour of methyl alcohol is found to be less than the expansion x_L produced by immersion in the liquid. The expansion is proportional to the surface energy lowering of the charcoal, and this relation is confirmed by comparing $\left(x_L - T\frac{dx_L}{dT}\right)$ with the measured heat of wetting. On immersion of the air-free charcoal the liquid wets all, or nearly all, of the adsorbing surface. From well-supported estimates, previously given, of the specific surface of the charcoal, it becomes possible to assign values to the free and total energy changes per cm.^{2} which accompany saturation and immersion.

The crystal structure of certain bridged palladium compounds.

By A. F. Wells. (*Communicated by J. D. Bernal, F.R.S.—Received* 26 *March* 1938.)

The crystal structures of the bridged compounds $[(CH_3)_3As]_2Pd_2Cl_4$ and its bromine analogue have been determined. The crystals of the tetrachloride from alcohol and from dioxane and those of the tetrabromide from dioxane are all isomorphous. A probable structure deduced from the optical properties, cell dimensions and symmetry was confirmed by Fourier projections using visually estimated intensities. The structure of the tetrabromide was investigated in detail, the configuration of the molecule being finally obtained from a section of the three-dimensional electron density distribution. The bridged molecule is found to be planar with the configuration



$$\begin{array}{c} (CH_3)_3As \\ Br \end{array}\!\!\!>\!Pd\!<\!\!\begin{array}{c} Br \\ 86^\circ \\ Br \end{array}\!\!\!>\!Pd\!<\!\!\begin{array}{l} Br \\ 86^\circ \\ As(CH_3)_3 \end{array}$$

The interatomic distances are: Pd–Br 2·45 and Pd–As 2·50 ± 0·05 A. The molecules are stacked in columns along 4_2 axes in the space group I $4/m$, and the columns of molecules are held together by very weak van der Waals forces. The packing of the molecules in this way leaves tunnels through the structure. These remain empty in certain circumstances, as, for example, when the tetrachloride crystallizes from alcohol. On crystallizing from dioxane, however, this compound takes up dioxane of crystallization presumably owing to the fact that the diameter of the dioxane molecules is approximately the same as that of the holes in the structure. The introduction of the solvent is accompanied by a small increase in the cell size and a slight reorientation of the molecules of the palladium compound.

The paramagnetic magneton numbers of the ferromagnetic elements.

By W. Sucksmith and R. R. Pearce. (*Communicated by A. M. Tyndall, F.R.S.—Received* 28 *March* 1938.)

A method is described for measuring magnetic susceptibilities in a controlled atmosphere or *in vacuo* at temperatures up to 1500° C. The special precautions required to prevent solid diffusion and minimize evaporation in measurements on metals are discussed. Accurate measurements on the susceptibilities of the ferromagnetic elements, hitherto confined to nickel, are extended to cobalt and iron. The magneton numbers for both these elements are deduced to an estimated accuracy of 1–2 % from the experimental data. It is shown that the existing theory is inadequate to explain the new results obtained.

Electromagnetic induction in non-uniform conductors, and the determination of the electrical conductivity of the earth from terrestrial magnetic variations.

By A. T. Price and B. N. Lahiri. (*Communicated by S. Chapman, F.R.S.—Received* 28 *March* 1938.)

The results of previous investigations by Chapman and Price of the induced fields and current distributions, associated with the magnetic daily and storm-time variations, suggest that more precise information as to the distribution of electrical conductivity (κ) within the earth, might be obtained by considering electromagnetic induction in a non-uniform sphere. The general theory for any non-uniform conductor is here considered, and the formal solution for any conductor with spherical symmetry is obtained. Detailed formulae for the induced field and current distribution, in the special case when $\kappa = k\rho^{-m}$, where k and m are constants, are obtained and applied to the terrestrial magnetic variations. The results obtained support the view, expressed by Chapman and Price, that there is a considerable increase of κ with increasing depth, beyond 150 km. It seems, however, that the really important increase in κ takes place at about 700 km. depth, beyond which κ is at least as great as 10^{-11} e.m.u., while above this depth the mean conductivity may be of the same order as for rocks on the earth's surface (10^{-16} or 10^{-15} e.m.u.). This suggests that there is some change in the composition of the earth (e.g. to a more metallic content) at a depth of about 700 km.; seismological evidence appears to indicate that such a transition occurs at a somewhat greater depth. The results also show that there is an effective distribution of κ at or near the surface of the earth, and it seems most probable that this represents the influence of the relatively highly conducting oceans. The induced currents do not penetrate appreciably beyond a depth of about one-fifth of the earth's radius, so that the knowledge of κ afforded by the daily variations and the storm-time variations will be restricted to an outer shell of this thickness.

The photochemical polymerization of methyl acrylate vapour. By H. W. MELVILLE. (*Communicated by E. K. Rideal, F.R.S.—Received 28 March 1938.*)

The photopolymerization of methyl acrylate vapour has been studied at pressures up to 60 mm. and at temperatures between 20 and 150° C. Polymerization occurs almost quantitatively, giving a dense cloud of polymer in the gas phase when about 10 quanta are absorbed/c.c./sec. at 2537 A, the quantum yield ranging from unity to 5000 depending on the temperature and intensity. At shorter wave-lengths the molecule is dissociated to hydrogen and propiolic ester. Growth of the polymer ceases when two growing polymers interact with each other. The temperature of this latter process is so large that the polymerization has a negative temperature coefficient.

The reaction is very sensitive to inhibitors especially to oxygen and to a lesser extent butadiene. By controlled use of inhibitors, a direct measure of the energy of activation of the propagation reaction has been made: it amounts to 4 k.cal. At high oxygen pressures, oxygen exerts a positive catalytic effect believed to be due to the mercury sensitized production of O atoms.

The molecular weight of the polymer has been estimated by measuring the ratio of acrylate to butadiene molecules used up in the inhibited reaction under a given intensity of illumination. Hence by calculation the molecular weight of the product in the normal reaction is easily found.

This behaviour of acrylate is in marked contrast with that exhibited by methyl methacrylate, the long life-time of the latter polymer being absent in the acrylate. Like the methacrylate the acrylate grows by the double bond mechanism and the differences in behaviour of these two molecules is accounted for by the theories advanced in this and previous papers.

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable. The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form. These abstracts will be issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings".

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19 May 1938

The investigation of electron lenses. By O. Klemperer and W. D. Wright. (*Communicated by W. H. Eccles, F.R.S.—Received* 29 *March* 1938.)

The paper describes two methods of deriving the optical constants and spherical aberration of electrostatic lens systems. In the first, the electrical field is imagined to be divided into a series of equipotential surfaces at each of which there is a finite change of potential. To a first approximation these fields are spherical close to the axis and the normal optical ray tracing formulae can be used to compute the paths of electrons through the system. Results are reported for a two-tube lens for various focusing ratios, the two tubes being of equal diameter. The aberration contributions of the different refracting surfaces to the final aberration can be evaluated and the curvature of field can also be determined by this means. In the second method, the Hartmann test used in glass optics has been applied to the experimental investigation of electron lenses. Narrow pencils of electrons pass through a suitable diaphragm and their course when focused by the lens under test is found by measurements of their intersections with a fluorescent screen placed at appropriate points along the axis. The design of an electron gun suitable for these tests is described, and results for the two-tube lens are given for voltage ratios from 2·5:1 to 15:1.

Trypanocidal activity and chemical constitution. By G. M. Dyson, T. H. Fairbrother, F. A. Mason and A. Renshaw. (*Communicated by H. S. Raper, F.R.S.—Received* 29 *March* 1938.)

The physiological action of the complex ureas has been shown to be very specific. The smallest alteration in chemical constitution such as the omission of a methyl group seriously alters the trypanocidal activity. The object of this research has been to investigate the variations in trypanocidal activity produced by certain changes in the typical urea structure; it has been shown that the accumulation of three sulphonic acid groups in suitable orientation in the terminal part of the molecule is necessary for the development of trypanocidal activity; further that the intermediate components must not depart appreciably from the *m*-amino-*p*-benzoyl-*m*-amino-*p*-toluyl structure. More latitude is allowable in the structure of the bridge linkage, which may have other configurations without complete extinction of trypanocidal activity. Whilst the urea bridge gives the best results, the carbazide linkage is almost equally suitable, although precautions must be taken not to destroy it with the formation of a urazine structure, during preparation.

Numerous examples of the variations in the typical structure of these ureas have been prepared and their physiological properties examined; they comprise the systematic alterations of terminal, intermediate and bridge linkages and also the introduction of halogen substituents in the aromatic rings. In addition, for purposes of comparison, a number of dyestuffs of the trypan red and blue series have been prepared and examined.

Experiments made with a half-Pitot tube. By G. I. TAYLOR, F.R.S. (*Received* 31 *March* 1938.)

The half-Pitot tube was invented by Stanton for measuring the tangential stress at the surface of a solid past which fluid is flowing. Stanton calibrated his tubes by inserting them in a pipe through which air was flowing in steady motion. He found that the pressure in his tubes decreased as their size diminished, but he did not attain the limiting condition when the half-Pitot is very small, nor did he express his results in non-dimensional form so that observations made with different sized tubes could be compared.

The present paper describes measurements made with a half-Pitot tube in a stream of glycerine. It is found that when the size of the tube is very small the pressure in it is 1·2 times the tangential stress acting on the surface through which it projects. The results, together with those of Stanton, are expressed in non-dimensional form.

Summary of electro-magnetic induction in moving bodies. By E. G. CULLWICK. (*Communicated by Sir Frank Smith, Sec.R.S.—Received* 24 *March* 1938.)

The paper has particular reference to the problems of unipolar induction.

The resultant electric field at a point is given in terms of: (*a*) the positions of electric charges, (*b*) the magnitude and velocity of each component of the magnetic field, on the assumption that each component is to be regarded as sharing the motion of its source, and (*c*) the accelerations of electric charges.

It is held that this method of calculation is consistent with the restricted theory of relativity, and removes the implication of a fixed aether from the usual relativity modifications of the Maxwell-Lorentz equations. It is further shown that open-circuit experiments on rotating magnets and solenoids are all consistent with the general validity of the moving-field theory, in spite of much argument to the contrary, and that indeed the balance of the evidence seems to be against the universal validity of the fixed-field Maxwell-Lorentz theory.

The hyperfine structure of the Zeeman components of the resonance lines of sodium.

By D. A. Jackson and H. Kuhn. (*Communicated by F. A. Lindemann, F.R.S.—Received* 4 *April* 1938.)

The hyperfine structure of the Zeeman π components of the two resonance lines of sodium was observed by means of the absorption in an atomic beam, the high resolving power instrument being a double etalon with separations of 2 and 8 cm. In strong fields, each Zeeman component was found to possess four hyperfine structure components, proving with certainty that the nuclear spin of sodium is $\frac{3}{2}$.

The alteration in the spacings of the lines as the field strength was changed from 3060 to 790 gauss was found to be in excellent agreement with that predicted by quantum mechanics.

A direct determination of the hyperfine structures of the terms $3S_{\frac{1}{2}}$ and $3^2P_{\frac{1}{2}}$ was made from the complete resolution of the line 5896.

Rotational analysis of the first negative band spectrum of oxygen.

By T. E. Nevin. (*Communicated by A. W. Conway, F.R.S.—Received* 6 *April* 1938.)

The (1, 0), (0, 0) and (0, 1) bands of the first negative system of O_2^+ have been photographed in the second order of a 21 ft. grating. Each of the bands has been analysed into forty branches, the transition involved being $^4\Sigma \rightarrow {}^4\Pi$. The fine structure of the $^4\Sigma$ level agrees with the theoretical structure predicted by Budó. The $^4\Pi$ level is inverted. The structure of the level does not agree with that to be expected on theoretical grounds from formulae given by Brandt and Budó. It is suggested that this disagreement is caused by a perturbation similar to that observed by Bulthuis and Coster in the $A^2\Pi$ level of CO^+.

Studies on the nature of the amphibian organization centre. VII.

By C. H. Waddington. (*Communicated by Sir Frederick Hopkins, F.R.S.—Received* 15 *January* 1938.)

1. Certain carcinogenic and oestrogenic substances, and other related compounds, were tested for evocator capacity by implantation in the blastocoel of newt gastrulae.
2. The substances were implanted in the form of suspensions in coagulated egg albumen. In an extensive series of controls, no inductions were obtained with albumen alone.

3. In the experimental series, a large number of inductions were obtained, the eggs showing much greater reactivity than in previous years. The results are summarized in Table I.
4. Inductions were obtained with a considerable number of substances, and no hypothesis can yet be made as to the relation between chemical structure and evocator power.

The origin and developmental mechanics of the avian sternum.

By HONOR B. FELL. (*Communicated by E. S. Goodrich, F.R.S.—Received* 21 *April* 1938.)

The development of the avian sternum was studied in relation to three main problems: (1) the origin of the sternum, (2) its anatomical differentiation and (3) the union of the sternal plates. The normal development of the budgerigar sternum was investigated, and points which could not be settled by ordinary morphological methods were studied experimentally by tissue culture. It was shown that the presumptive sternal tissue is not derived from either the ribs or the coracoid although it develops in close association with the latter; its position in the lateral body wall of the 4-day embryonic fowl was defined experimentally. The anatomical development of the sternum was found to be controlled largely by intrinsic factors, but the results suggested that extrinsic factors probably serve (*a*) to provide the optimal conditions for the expression of potencies inherent in the rudiment, and (*b*) to maintain the normal anatomical structure after it has once differentiated. The shape of the sternum depends partly on the relationship of growth rate to differentiation rate, and artificial disturbance of this relationship produces abnormalities of form. Evidence was obtained indicating that union of the sternal plates is due primarily to the active, amoeboid migration of adjacent undifferentiated cells towards the mid-line, and secondarily to shrinkage and degeneration in the mid-ventral body wall.

Investigation into the relation of shower frequency to general cosmic-ray activity.

By L. JÁNOSSY. (*Communicated by P. M. S. Blackett, F.R.S. —Received* 12 *April* 1938.)

The frequency of cosmic-ray showers was observed at sea level and under 30 m. of clay (60 m. of water equivalent). The shower frequencies at the two levels were compared with the corresponding vertical intensities. Part of the experiments were carried out with layers of light material close above the lead in order to maintain similar geometrical conditions for the shower production at both levels. The marked observed effect of the stone on the transition curve can be understood as due mainly to the spatial association of the shower-producing electrons emerging from the stone rather than to filtration. The relative shower intensity, i.e. (shower frequency/vertical intensity), of the showers producing the first slope of the transition curve was found to be mainly the same at sea level and underground; on the other hand the shower frequency due to showers producing the tail of the transition curve increases relatively with depth. The conclusion was drawn that most of the showers at sea level and underground are due to secondaries of some kind produced by the penetrating particles, and that consequently relatively few can be due to primary electrons.

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable. The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form. These abstracts will be issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings".

3 June 1938

The absorption spectra of the halogen acids in the vacuum ultra-violet.

By W. C. Price. (*Communicated by R. G. W. Norrish, F.R.S.—Received 20 April* 1938.)

The absorption spectra of hydrogen iodide, bromide and chloride have been investigated in the vacuum ultra-violet. The bands observed are due to the excitation of a non-bonding $p\pi$ electron and are the strong resonance bands approaching the $^2\Pi_{1\frac{1}{2},\frac{1}{2}}$ states of the molecular ion. They start at 1762, 1491 and 1331 A for hydrogen iodide, bromide and chloride respectively. In the case of hydrogen iodide a well-developed Rydberg series of bands was found approaching the $^2\Pi_{\frac{1}{2}}$ state of HI^+, the limit of which corresponded to $10{\cdot}990 \pm 0{\cdot}005$ V. It was not possible to establish with certainty Rydberg series going to $^2\Pi_{1\frac{1}{2}}$ of HI^+ or to any of the $^2\Pi$ states of HBr^+ or HCl^+. However, from correlations made amongst the bands of the different acids and with the corresponding transitions in the methyl halides, the mean ionization potentials (i.e. $HX:{}^1\Sigma \rightarrow HX^+:{}^2\Pi$) are determined to be 10·71, 12·04 and 12·84 V for hydrogen iodide, bromide and chloride respectively, the probable error being about a tenth of a volt.

The influence of magnetic fields on persistent currents in superconductors.

By K. C. Mann, H. Grayson Smith and J. O. Wilhelm. (*Communicated by A. S. Eve, F.R.S.—Received 20 April* 1938.)

Using the method of persistent currents to detect the onset of resistance of cylindrical specimens in transverse and longitudinal fields, the critical fields H_T and H_L (marking the appearance of this resistance) have been investigated. H_L for spectroscopically pure tin agrees very well with published critical fields, but is slightly higher than these at lower temperatures. For spectroscopically pure tin, $\frac{H_T}{H_L}$ is a constant 0·58 independent of temperature. Supplementary measurements carried out on a single crystal of "chemically pure" tin give longitudinal critical fields H_L considerably lower than for the spectroscopically pure tin, and the characteristic ratio shows a linear dependence on temperature. It is concluded from these results and from the results of other workers, that the dependence of the ratio on temperature is due to some other factor than crystal structure or purity.

The critical fields for very pure tin to which was added small amounts of both supraconducting and non-supraconducting impurities were also investigated. In all cases, H_L for those impure specimens is lower than the value for pure tin, but the magnitude of the effect depends upon the type of impurity used. The effect of impurities on H_T is very marked. Supraconductors raise this critical field, while the non-supraconductor lowers it. The characteristic ratio for tin-bismuth is about the same as for pure tin, while for tin-lead and tin-thallium, the ratios are roughly 0·75 and 0·65 respectively.

The experiments definitely show that pure tin in the intermediate state can carry without resistance a considerable, though limited current. It is concluded that impurities affect this current-carrying capacity.

Time effects were also observed with all specimens as they passed into the intermediate state. These were caused by the dying away of persistent currents which had been induced in the circuit above the critical value that the circuit could carry without showing resistance. These effects also differed with the purity of the specimen, showing that the resistance of the intermediate state is affected by impurities.

A new form of resorcinol. Part I. Structure determination by X-rays.

By J. Monteath Robertson and A. R. Ubbelohde. (*Communicated by Sir William Bragg, P.R.S.—Received 23 April 1938.*)

The structure of the dense, high-temperature modification of resorcinol has been fully determined by means of a quantitative X-ray investigation. The structure is characterized by a more compact and parallel arrangement of the molecules than in ordinary resorcinol, which is a rather open arrangement. The benzene rings are apparently regular hexagons to within 0·05 A, but the OH groups are definitely displaced from the symmetrical positions, and one of them at least is also displaced out of the plane on the ring. The carbon-oxygen distances are 1·36–1·37 A, as in ordinary resorcinol. The orientation of the molecule in the crystal and the atomic co-ordinates are given. Hydroxyl bonds of length 2·70 and 2·75 A connect neighbouring molecules in the crystal, but the angles between these bonds are further distorted from the tetrahedral value than in ordinary resorcinol. All other intermolecular distances are greater than 3·4 A.

A new form of resorcinol. Part II. Thermodynamic properties in relation to structure.

By J. MONTEATH ROBERTSON and A. R. UBBELOHDE.

(*Communicated by Sir William Bragg, P.R.S.—Received* 23 *April* 1938.)

The anomalous temperature relationship between the polymorphs of resorcinol is discussed in the light of the crystal structures. The open α structure is maintained by hydroxyl bonds orienting the rigid resorcinol molecules. Transition to the denser β structure, which resembles that of many hydrocarbons, is associated with a distortion of the hydroxyl bonds, and a deformation of the resorcinol molecule which may involve a Mills Nixon effect.

Experiments are described on the preparation of α and β resorcinol, on the transition between the two forms, and on the heat of transition. The transition point of $C_6H_4(OH)_2$ is about 2° lower than that of $C_6H_4(OD)_2$, and the change from one form to the other is considerably more rapid in the deuterium compound. The heat of transition from α to β resorcinol is 220 ± 50 cal./mol. Experiments on the volume change from (supercooled) liquid resorcinol to either crystal structure show that there is only a small expansion on passing from the open α structure to the liquid. Experiments on the transition between two polymorphs of pentaerythritol show a normal temperature relationship for this substance.

The electrical response of the auditory mechanism in cold-blooded vertebrates.

By E. D. ADRIAN, F.R.S., K. J. W. CRAIK and R. S. STURDY.

(*Received* 25 *April* 1938.)

The effects of sound on the ears of some cold-blooded vertebrates have been analysed by recording the electric changes set up in the inner ear and in the auditory nerve. The inner ear potentials (the equivalent of the Wever and Bray effect in the mammal) can be recorded in animals without a cochlea and seem to be due to the movement of polarized membranes. As they show the mechanical vibrations transmitted to the inner ear they can be used to compare the efficiency of different types of middle ear. Thus the alligator (with a membranous ear drum) gives an inner ear response to sounds of higher frequency than the tortoise (drum covered by external skin), though at low frequencies the tortoise ear is as sensitive. In fish the inner ear reacts to mechanical vibration although there is no middle ear.

A change of temperature has little effect on the performance of the middle ear or on the inner ear potentials, but the frequency at which the auditory nerve fibres can respond will be limited by the refractory period and therefore by the temperature. At 10° C. the nerve fibres in the frog and tortoise will not signal frequencies much above 200 per sec., whereas at 20° C. they will signal 400–500. These results emphasize the value of a constant high temperature for the receptor mechanism for sound.

The determination of the meteorological conditions of the atmosphere by the use of radio-sounding balloons. By H. A. Thomas. (*Communicated by Sir George Simpson, F.R.S.—Received* 26 *April* 1938.)

Existing methods of exploring the atmosphere by radio-sounding balloons are reviewed briefly and it is explained that arrangements involving variation of radio frequency are not likely to be satisfactory due to the wide frequency band required and the chances of appreciable interference. Furthermore, methods involving signalling codes are complex and expensive, and the delineation of the signals at the receiver presents considerable difficulty.

The essential features of an original method of exploration are described; in this method, a signal of fixed radio frequency is employed and each meteorological instrument produces a continuous variation of modulation frequency. Calibration of the meteorological parameters in terms of frequency can be made independently of the radio transmitter and measurement of these audio frequencies at the receiver is comparatively easy. The pressure and temperature measuring instruments are both arranged to produce variation of modulation frequency without the use of mechanical linkages. The cost of the apparatus is comparatively low and reproduction in large quantities is possible.

The results obtained from a number of experimental ascents are analysed and it is shown that a high degree of reliability and accuracy is obtainable. Using the present balloon, observation of both pressure and temperature up to an altitude of about 10 kilometres is obtained, the accuracy of the pressure and temperature determinations being about 5 mb. and 1° C. respectively.

The effect of anterior pituitary extracts on established lactation in the cow. By S. J. Folley and F. G. Young. (*Communicated by A. S. Parkes, F.R.S.—Received* 26 *April* 1938.)

Single injections of a crude anterior pituitary extract, and of prolactin and thyrotrophic preparations obtained from fresh ox anterior lobe, into cows in declining lactation, resulted in each case in a substantial increase in the milk yield for 5 or 6 days. Only slight changes were found in the composition of the milk.

A single injection of prolactin prepared from desiccated ox anterior lobe administered in a dose equivalent, as regards crop gland stimulation, to the dose of prolactin from fresh gland, had no effect on the milk volume or composition. Likewise a single injection of a dose of the thyrotrophic fraction prepared from dried gland, equivalent to the dose previously used of the thyrotrophic fraction from fresh gland as regards ability to cause enlargement of the thyroid gland, had no effect on the milk volume or composition.

The daily injection of very large doses of prolactin prepared from dried gland resulted in a substantial and prolonged increase in milk volume, with a rise in milk lactose content accompanied by a fall in the milk chlorides.

The thyrotrophic preparation from fresh gland, although lactogenic in the lactating cow, possessed no detectable pigeon crop gland stimulating activity. The prolactin preparation from dried gland, although possessing substantial pigeon crop gland stimulating activity, failed to exhibit lactogenic activity when a single dose was injected into a lactating cow. The relative lactogenic activities of the extracts used are paralleled, not by their activities in causing stimulation of the pigeon crop gland, but by their relative glycotropic (anti-insulin) activities.

There seems to be no satisfactory evidence that a single "lactation hormone" is secreted by the anterior pituitary gland and it is suggested that use of this term should be discontinued.

The mechanism of chain breaking in the decomposition of ethane. By C. N. HINSHELWOOD, F.R.S., and J. E. HOBBS. (*Received* 27 *April* 1938.)

In the decomposition of ethane there are chains which appear normally to be broken by the recombination of radicles, but, in the presence of nitric oxide, by reaction between it and the radicles. The equation for the curve showing the rate of reaction as a function of nitric oxide concentration contains constants which depend differently upon the ethane concentration according to the mechanism assumed for the normal recombination of the radicles. Experimental investigation gives evidence that the predominant mechanism for chain ending in the absence of nitric oxide is a ternary collision between two radicles and an ethane molecule, but that binary collisions are also in some degree effective. This result is discussed.

Reaction chains in the thermal decomposition of hydrocarbons. A comparison of methane, ethane, propane and hexane. By C. N. HINSHELWOOD, F.R.S., and J. E. HOBBS. (*Received* 27 *April* 1938.)

A comparison has been made of the inhibition by nitric oxide of the thermal decomposition of methane, ethane, propane and hexane. The mean chain length, as defined by the ratio of the rates of the uninhibited and fully inhibited reactions, shows no marked variation as the series is ascended, has values from 2 to 15 according to conditions, and, for a given hydrocarbon, decreases as the initial pressure increases. The curves showing the decrease in rate as a function of the nitric oxide concentration can be expressed in terms of a simple theory of chain breaking. The mean chain length depends upon the relative values of three activation energies, namely those of radicle formation, of decomposition by internal rearrangement, and of chain propagation. Approximate evaluation of the last shows that it tends to decrease with the higher hydrocarbons.

A study of sensitized explosions. II. Ignition phenomena in mixtures of carbon monoxide and oxygen sensitized by hydrogen. By E. J. Buckler and R. G. W. Norrish, F.R.S. (*Received* 27 *April* 1938.)

In extending the research of other workers on the catalytic influence of hydrogen on the oxidation of carbon monoxide, it has been found that ignition in mixtures containing hydrogen occurs at temperatures more than 150° below those of the pure gases, and is attended by complete combustion when the concentration of hydrogen exceeds about 0·5 %. The region of sensitized ignition is bounded by a lower pressure limit which was not investigated in detail, and an upper pressure limit the dependence of which on temperature and hydrogen concentration was determined by the slow withdrawal of sensitized mixtures from the reaction vessel up to the point of explosion.

Mixtures are stable above the upper pressure limit obtained by the withdrawal method, but may be ignited by sudden compression. A study of the minimum degree of compression required to produce inflammation in mixtures containing different inert gases proved that although the initial presence of free radicals containing hydrogen was essential, the primary cause of ignition was the momentary rise of temperature accompanying sudden compression.

It has also been found that mixtures containing a suitable percentage of hydrogen admitted suddenly to an evacuated reaction vessel ignite when the final pressure is only slightly greater than the upper limit determined by the withdrawal method, but for higher final pressures may be introduced without inflammation occurring. However, ignition reappears when the final pressure is further increased to values above 100 mm. Hg, due to approximately adiabatic compression of the first portions of gas to enter the reaction vessel. Owing to the momentary rise of temperature in the compressed portion of the mixture, ignition spreads out from reaction centres formed by chain branching processes operative during the early stages of admission.

A study of senzitized explosions. III. The kinetics of ignition of carbon monoxide and oxygen sensitized by hydrogen. By E. J. Buckler and R. G. W. Norrish, F.R.S. (*Received* 27 *April* 1938.)

It has been found that mixtures of carbon monoxide and oxygen containing only traces of hydrogen resemble oxy-hydrogen mixtures in that both ignite over the same range of temperatures, and both possess upper pressure limits of ignition not markedly affected by change of vessel diameter, which vary in the presence of inert gases in a manner suggesting that the processes responsible for multiplication of chain carriers may be prevented by collisions of the second kind with other molecules. In mixtures of $2CO + O_2$ of constant $CO : H_2$ ratio the relation between $1/T$ and $\log P$ (P being the pressure limit of ignition at $T°$ abs.) is linear when the concentration of hydrogen is less than about 2 %, with a slope corresponding to an apparent energy of activation of 13,300 cal., approximately one-half the corresponding figure given for mixtures of $2H_2 + O_2$, while for mixtures of $2CO + O_2$ containing higher percentages of hydrogen the curves show a variable slope. These facts suggest that carbon monoxide introduces addition chain-ending processes into the mechanism of the hydrogen-oxygen reaction. A similar value for the apparent energy of activation is obtained in mixtures of $2CO + O_2$ containing 0·5 % of deuterium, while the ratio of the values of the pressure limits of ignition of mixtures sensitized by the same percentage of hydrogen and deuterium respectively becomes constant at 1·42 when the concentration of sensitizer is below about 2 %.

The catalytic effect of hydrogen on the combustion of carbon monoxide, and the differences in kinetic characteristics between this reaction and that in mixtures of pure hydrogen and oxygen can be completely described by the introduction of two additional elementary reactions $O + CO + M = CO_2 + M$ and $OH + CO = CO_2 + H$ into the mechanism for the ignition of mixtures of $2H_2 + O_2$ proposed by Kassel and Storch (1935) and modified by von Elbe and Lewis (1937).

The origin of the erythema curve and the pharmacological action of ultra-violet radiation. By J. S. Mitchell, with an appendix by C. B. Allsopp and J. S. Mitchell. (*Communicated by E. K. Rideal, F.R.S.—Received* 2 *May* 1938.)

The measurements of the erythema curve and the relevant known facts of the reaction of the human skin to ultra-violet light are reviewed. The possibility of a photochemical mechanism is discussed and developed theoretically.

Quantitative evidence is presented to show that the erythemal response is due to photochemical decomposition of typical proteins in the stratum mucosum by the light transmitted through the stratum corneum; this latter behaves as an inert scattering and absorbing screen. The photochemical changes are limited to the stratum mucosum by the distribution of pigment. The latent period is, at least in part, the time required for the diffusion of active photo-decomposition products to the minute vessels.

The form of the erythema curve is calculated, within the limits of experimental error, from absorption measurements of a typical protein and of the human stratum corneum. The calculated mean value of the wave-length of maximum erythemal efficiency is 2975 (± 15) A, in close agreement with that observed. The known minimal erythema dose is consistent with the formation of photodecomposition products *in vitro* in concentration of a reasonable order of magnitude.

It is suggested that the H-colloid may be a proteose formed by oxidative photolysis of proteins.

The relationship of erythemogenesis to pigmentation and carcinogenesis by ultra-violet radiation is briefly discussed.

The function of the ampullae of Lorenzini, with some observations on the effect of temperature on sensory rhythms. By A. Sand. (*Communicated by S. W. Kemp, F.R.S.—Received* 2 *May* 1938.)

The histological structure of the ampullae of Lorenzini is still in dispute, though there is strong evidence that they are not neuromasts. The central connexions of their innervation have not been thoroughly studied. Their functional activity, as revealed by the present oscillographic investigation, is entirely distinct from that of lateral-line organs. For these reasons their unqualified inclusion in the acusticolateralis system is unwarranted.

Like acustico-lateral receptors they possess an autonomous rhythm of activity, but in contrast with the former, they are unaffected by any form of mechanical stimulation. Their sensory sensitivity is confined to thermal stimulation.

Their responses to temperature changes are highly peculiar. They respond to cooling by an acceleration, to warming by an inhibition of the rhythm of impulse discharge. Such responses have been shown to occur throughout the physiological temperature range, from 3 to 20° approximately, though it is probable that they are most sensitive in the region 10–15°. In this region clear responses have been recorded to temperature differences of one or two-tenths of a degree.

After a response to a temperature change, adaptation occurs, and a regular slow rhythm of discharge is re-established. Such adapted frequencies plotted against temperature yield the familiar type of temperature curve with a positive temperature coefficient.

Lateral-line and stretch receptors of *Raja* show a similar paradoxical behaviour with respect to temperature changes, but on an entirely different scale. Stimuli of the order of 10° are required to produce an effect, and their maximal responses, compared with those of the ampullae, are minute. This is interpreted as meaning that the paradoxical effect of temperature changes is common to sensory rhythms in general, and possibly to some tonic muscles as well, but that in the ampullae of Lorenzini the mechanism has become specialized and sensitized for the performance of a specific thermosensory function.

The general form of a hypothesis to account for the paradoxical temperature effect is suggested. It involves the assumption of underlying reactions with different time factors of kinetic equilibration.

No cutaneous thermosensory endings have been found in rays, and it is concluded that the ampullae of Lorenzini are the only temperature receptors of elasmobranch fishes.

An interferometric wave-length comparison of the red cadmium radiation emitted by different sources. By D. V. Gogate and W. Ewart Williams, *King's College, London.* (*Communicated by O. W. Richardson, F.R.S.—Received* 5 *May* 1938.)

A comparison is made between the wave-lengths of the red cadmium line ($\lambda = 6438$ A) emitted by a G.E.C. Osira cadmium lamp and a Schüler hollow cathode discharge by photographing the Fabry-Pérot fringes due to the two sources side by side in the same horizontal plane by means of a specially designed plate holder. Interferometer gaps up to 10 cm. were used and the greatest difference recorded was less than 1 part in 100×10^6 or 0·00006 A.

ABSTRACTS

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16 June 1938

Sorption of polar and non-polar gases by zeolites. Parts I and II. By Richard M. Barrer. (*Communicated by E. K. Rideal, F.R.S.—Received 2 May* 1938.)

The sorption on five different zeolites representative of the three types of zeolite structure—three-dimensional networks, fibrous, and laminar—has been studied for helium, hydrogen, argon, nitrogen, and ammonia. The more simple types of equilibria obtained with the permanent gases were used to interpret the more complex equilibria observed with ammonia. In the case of the permanent gases, only the three-dimensional networks (chabasite and analcite) sorbed gas within the dehydrated lattice. The fibrous and platy zeolites underwent a reorganization of ions around the water-bearing channels which was sufficient to exclude even helium (diameter 2·5 A). How marked the change was, was indicated by calculations of the potential within one of these zeolites (natrolite) which showed that even if methane (4·16 A diameter) were to replace water in an *otherwise unchanged* natrolite it would do so with a high sorption potential. The sorption of ammonia, however, occurred in fibrous and platy zeolites as well as in those of three-dimensional network type. A complete gradation in behaviour was found in the series:

NH_3—chabasite, NH_3—analcite, NH_3—heulandite, NH_3—natrolite,

the first giving a simple sorption system, and the last an ammoniate, the other two being intermediate. It was observed that a new phase tended to appear when the act of sorption required a reorganization of the lattice in order to accommodate the molecule sorbed. It was possible, however, so to alter the zeolite by outgassing, that not even ammonia would penetrate (NH_3—scolecite); and also to retard the resorption rate greatly by a prolonged heating while not completely inhibiting it (NH_3—heulandite). Kinetic studies of sorption showed that two types of ammonia uptake occurred: (1) A diffusion of NH_3 down a concentration gradient (NH_3 in three-dimensional networks). (2) A diffusion of ammoniate-anhydrous zeolite interfaces (NH_3—natrolite); this latter process was auto-catalytic in its initial stages. Sorption velocities were most rapid in chabasite and analcite—in the latter instance contrary to Tiselius' earlier finding.

The sorption heats were measured for

NH_3, H_2, Ar, N_2	—chabasite,	NH_3, H_2, Ar	—scolecite,
NH_3, He, H_2, Ar, N_2	—analcite,	NH_3	—heulandite,
NH_3, Ar	—natrolite,		

in many instances as a function of both quantity of gas sorbed and of temperature. The temperature coefficient of the heats for the permanent gases was negligible, and the heats tended to diminish with rising temperature for ammonia, and to diminish with increasing amount sorbed for all the gases.

The apparent saturation maxima diminished nearly linearly with the absolute temperature for all the gases.

The results were compared and contrasted with some previous data.

On the stability of a viscous liquid between rotating coaxial cylinders.

By J. L. Synge. *(Communicated by S. Goldstein, F.R.S.—Received 5 May 1938.)*

For a small disturbance (independent of the azimuthal angle Φ) of the steady motion of a liquid between rotating coaxial cylinders, the equations of motion for cylindrical co-ordinates r, Φ, z may be written

$$\left(\frac{\partial}{\partial\tau}-\Phi\right)\Phi\psi=-2R\left(a+\frac{b}{\xi^2}\right)r_1\frac{\partial\eta}{\partial\zeta},$$

$$r_1\left(\frac{\partial}{\partial\tau}-\Phi\right)\eta=2Ra\frac{\partial\psi}{\partial\zeta},$$

$$\Phi=\frac{\partial^2}{\partial\xi^2}+\frac{1}{\xi}\frac{\partial}{\partial\xi}+\frac{\partial^2}{\partial\zeta^2}-\frac{1}{\xi^2},$$

where r_1, r_2 are the radii of the cylinders ($r_2>r_1$), ω_1, ω_2 their angular velocities ($\omega_1>0$), ν the kinematical viscosity,

$$a=\frac{1}{\omega_1}\frac{\omega_2r_2^2-\omega_1r_1^2}{r_2^2-r_1^2},\quad b=-\frac{1}{\omega_1}\frac{r_2^2(\omega_2-\omega_1)}{r_2^2-r_1^2},\quad R=\frac{\omega_1r_1^2}{\nu},$$

$$\tau=\nu t/r_1^2,\quad \xi=r/r_1,\quad \zeta=z/r_1.$$

η is the velocity of disturbance in the Φ-direction and ψ a function in terms of which the other two components of velocity are determined by means of the equation of continuity. On substituting

$$\psi=r_1f(\xi)e^{\sigma\tau+i\lambda\zeta},\quad \eta=ig(\xi)e^{\sigma\tau+i\lambda\zeta},$$

we obtain the ordinary differential equations

$$(L-\lambda^2-\sigma)(L-\lambda^2)f=-2\lambda R(a+b/\xi^2)g,$$

$$(L-\lambda^2-\sigma)g=-2\lambda Raf,$$

$$L=\frac{d^2}{d\xi^2}+\frac{1}{\xi}\frac{d}{d\xi}-\frac{1}{\xi^2},$$

with the boundary conditions $f=df/d\xi=g=0$ for $\xi=1$ and $\xi=\alpha$.

The first of these equations is multiplied by $\xi\bar{f}d\xi$ (the bar denoting the complex conjugate) and integrated over the range: the second equation is multiplied by $-\left(1+\frac{b}{a\xi^2}\right)\xi\bar{g}d\xi$ and integrated over the range. When the two resulting equations and their complex conjugates are added together, the imaginary part of σ disappears, and an expression is obtained for the real part of σ in terms of certain integrals. If $\omega_2 r_2^2 > \omega_1 r_1^2$, it is seen that the real part of σ is negative, and hence there is stability under this condition. This is in agreement with the theory and experiments of G. I. Taylor, but his detailed theory dealt only with the case where the distance between the cylinders is small. The present result is quite general, and is obtained without heavy calculations.

The approximate prediction of skin friction and lift. By N. A. V. PIERCY, J. H. PRESTON and L. G. WHITEHEAD. (*Communicated by E. F. Relf, F.R.S.—Received* 6 *May* 1938.)

Convenient methods are developed for predicting by successive approximation the laminar skin friction of cylinders according to any analytical assumption regarding the irrotational flow outside their boundary layers. These methods almost eliminate the need for computation till the stage is reached when remaining mathematical errors become small. The particularly simple third approximation to the solution of the boundary layer equations suffices for most purposes until breakaway is closely approached. Explicit formulae are obtained for the position of breakaway and for the skin friction of elliptic cylinders.

An appropriate assumption regarding the external flow, avoiding appeal to experiment, is derived by recognizing the existence of a wake to the extent of elongating the cylindrical profile from the points of breakaway to infinity. The theory of the modification is formulated and a rapidly convergent method of successive approximation developed for its application. It transpires that any reasonable assumption regarding the thickness and configuration of the rudimentary wake secures close agreement with experiment—in contrast to the familiar consequences of ignoring the wake.

A new criterion for determining the circulation round a lifting cylinder is proposed and, in illustration, the variation of lift with inclination of section is calculated from first principles for a given elliptic cylinder. The result agrees fairly with experiment, the "stall", in particular, being predicted with some accuracy.

Compared with earlier methods, those now advanced promise improved physical accuracy in the theoretical determination of skin friction, breakaway, lift and stall. Their analytical convenience will, it is hoped, facilitate investigations of great complexity.

Effusion phenomena in relativistic quantum statistics. By B. N. Srivastava. (*Communicated by M. N. Saha, F.R.S.—Received* 9 *May* 1938.)

In this paper the effusion phenomena in non-degenerate and degenerate matter have been investigated from relativistic mechanics. Matter obeying either Fermi-Dirac statistics or Bose-Einstein statistics has been considered. In § 1 non-degenerate matter has been considered, and the number of particles as well as the mass of matter effusing out has been calculated. The non-relativistic expressions are obtained as a limiting case. For $f = 10$ the departure from the value for a classical perfect gas amounts only to about 1 %. In § 2 the effusion has been calculated for a degenerate gas, degenerate in the sense of Fermi-Dirac statistics, both to a first approximation and to a second approximation. The non-relativistic formulae are obtained as a limiting case. In this case it is found that to a first approximation the effusion varies as $p^{\frac{4}{5}}$ and is independent of T, but on working to a second approximation a slight dependence on temperature is observed. The average mass of particles in the effusion stream is calculated.

The diffraction of X-rays by age-hardening aluminium copper alloys. By G. D. Preston. (*Communicated by P. M. S. Blackett, F.R.S.—Received* 10 *May* 1938.)

The paper contains a description of an effect observed in Laue and oscillating crystal photographs of an alloy of high purity aluminium with 4 % copper aged at room temperature and at 150° C. The effect is ascribed to the separation of copper on (100) planes of the aluminium lattice.

Rotatory dispersion in the amine series. Part IV. The optical activity of diamines. By W. C. G. Baldwin, M.A., Ph.D. (*Communicated by R. G. W. Norrish, F.R.S.—Received* 10 *May* 1938.)

Variations in Drude equations for the rotatory dispersions, and changes in specific rotation, which occur on neutralization, are utilized for the detection of induced dissymmetry in the amino-radical of optically active bases. When the lone pair of electrons of the nitrogen atom becomes bound in any way, the partial rotation associated with it disappears. An attempt is made to explain the behaviour of a few exceptional bases whose rotatory powers contain no contribution which can be attributed directly to the lone pair of electrons. Such bases do not give the large alteration in specific rotation which is usually produced by ionization.

The rotatory dispersion of crystalline ethylene-diamine sulphate is compared with that of l-propylene-diamine, l-diamino-*cyclo*hexane, and of their salts in solution. Measurements and analyses are given for these two optically active bases, as well as for their mono- and dihydrochlorides. The results are interpreted in terms of Boys's molecular model, and the absolute configuration of l-propylene-diamine is defined. In spite of statements in the literature to the contrary, the sign of the specific rotation of this base is actually reversed on ionization, and this experimental fact constitutes strong evidence in favour of the proposed model.

It is shown that the major contribution to the optical activity of l-propylenediamine may be attributed to the absorption bands associated with the two lone pairs of electrons in the molecule. Assuming the presence of an unchanging dextrorotatory nucleus common to all three ionic forms, an attempt to estimate the other contributions to the rotatory power and dispersion is made.

The absorption bands between 2300 A and 2000 A which are without exception common to all amines previously investigated are in the cases of l-propylene-diamine and l-*cyclo*hexane-diamine not optically active. The induced dissymmetry is centred at wave-lengths between 1900 A and 1400 A, and is associated with two groups of absorption bands in this region, one due to the lone pairs of electrons, and the other to the more saturated CN and NH linkages.

Investigations of infra-red spectra absorption of the CH_2 group in the region of 3μ. By J. J. Fox and A. E. Martin. (*Communicated by Sir Robert Robertson, F.R.S.—Received* 11 *May* 1938.)

The absorption of seven substances containing CH_2 groups has been investigated in the region of 3μ. Besides the aromatic CH bands found about $3{\cdot}27\mu$ when a benzene ring is present, two or more bands near $3{\cdot}4\mu$ are attributable to the CH_2 group.

A critical survey of the data available for ethylene enables us to explain the bands observed for CH_2 groups in more complicated molecules. When only one CH_2 group is contained in the molecule two bands are observed and these are identified as the CH valency vibrations in and out of phase, but when the number of CH_2 groups is increased, the coupling between them may increase the number of bands as in diozan, for example, where four strong bands are observed.

A satisfactory approximation to the molecules studied by us is found in a model of the formaldehyde type in which the CH_2 group is attached to a mass of similar magnitude. This model enables us to explain the observed frequencies provided that the CH force constant is $4{\cdot}52 \times 10^5$ dynes/cm., a value considerably lower than $5{\cdot}02 \times 10^5$ for ethylene and $5{\cdot}88 \times 10^5$ for acetylene. The important result is obtained that the mass to which the CH_2 group is attached and the various other force constants involved in compounds of the type investigated have very little effect on the positions of the CH_2 absorption bands in the 3μ region, except in rare cases involving strain of the bonds connecting the CH_2 groups to the rest of the molecule, e.g. fluorine.

The refraction and dispersion of air for the visible spectrum. By J. E. Sears, Junr. and H. Barrell. (*Communicated by W. L. Bragg, F.R.S.—Received* 11 *May* 1938.)

The refraction and dispersion of dry, CO_2-free air in the visible spectrum has been investigated, at temperatures between 12 and 31° C. and at pressures between 100 and 800 mm. of mercury, by means of an interference refractometer which employs Brewster's fringes produced by two 67 cm. Fabry-Pérot étalons. The results are used to provide a general interpolation equation from which definitive values of the refractive index may be calculated; the magnitude of the probable difference between a single observed value of refractive index and the value calculated from the equation is $\pm 0{\cdot}01_6 \times 10^{-6}$. The form of this equation, being derived from considerations based on the Lorenz-Lorentz relation between refractive index and density of transparent media and on D. Berthelot's representation of the characteristic behaviour of gases at low pressures, enables interesting comparisons to be made between the results of refractivity and compressibility measurements on air regarding the departure of the gas from Avogadro's law.

The effect of humidity on the refraction of atmospheric air has been determined from refractivity measurements on moist, CO_2-free air containing known amounts of unsaturated vapour.

No evidence has been found in favour of Tilton's suggested relationship between the refractivity of air and sunspot activity.

On the blood circulation and metabolism of Sabellids.

By H. MUNRO FOX, F.R.S. (*Received* 13 *May* 1938.)

Most of the blood vessels of sabellid worms are rhythmically contractile. The rates of contraction increase in the following order: crown capillaries, lateral vessels, ventral vessel, peri-intestinal sinus. The rate of contraction is more rapid in small than in large individuals.

There is evidence that the movement of the blood is a circulation.

When *Sabella* retires into its tube the contractions of the peri-intestinal sinus stop just before those of the crown vessels, while the lateral vessels do not cease contracting until later. The oxychlorocruorin in the stagnant blood is not completely reduced.

There are capillary blood vessels supplying body-wall glands but none in the muscles. The muscles must therefore obtain oxygen from the sea water and from the coelomic fluid, which is richly supplied with blood capillaries. The high total oxygen capacity of the blood and the high unloading tension of chlorocruorin may be correlated with this indirect oxygen supply to the muscles from the blood through the coelomic fluid.

Spirographis is negatively thigmotropic, negatively geotropic and positively phototropic. These tropisms involve permanent curvatures of the tube which are probably accomplished by softening the tube through digestion, together with secretion of elastic tube substance.

Spirographis deprived of its tube will live indefinitely in aerated water, but dies in water deficient in oxygen, although worms with their tubes remain healthy in this water.

Spirographis deprived of its tube and put into a glass tube, lines the glass tube with a secretion and lengthens it like a normal tube. Worms in these glass tubes remain healthy in water deficient in oxygen.

It can be seen from *Spirographis* in glass tubes that the worms in their tubes execute vigorous rhythmic body movements. These movements are unaffected by oxygen deficiency or carbon dioxide excess in the water.

The rhythmic body movements probably continually renew the water in the tube. These movements are feeble or absent in tubeless worms. It is probably for this reason that tubeless worms die in water deficient in oxygen, while worms with their tubes can live in water deficient in oxygen by continually renewing the water in contact with the body. Tubeless worms are viable in aerated water probably because they can obtain enough oxygen without body movements. These movements must be necessary to worms with their tubes, even when the surrounding water is aerated, because of the small lumen of the tube and the difficulty of renewing water through the anterior opening of the tube alone.

The tube has thus a second function in addition to being protective; it provides the mechanical stimulus for reflex rhythmic body movements necessary to the animal with its tube.

When *Spirographis* is removed from its tube, its oxygen consumption falls to three-quarters of the initial value on the same day, and to one-half on the following day, while its ammonia excretion falls on the day of removal from the tube to four-fifths of the initial value. This fall in metabolism must be due in part to the absence of vigorous rhythmic body movements in the tubeless worms.

When the crown of *Spirographis* is amputated, the rate of oxygen consumption of the worm falls to one-third of the value for the intact worm. This is true both of worms with their tubes and of those deprived of tubes.

The rate of oxygen consumption of the isolated crown of *Sabella* is 2½ times that of the whole worm, but this high rate of metabolism of the crown is not sufficient to account for the fall in oxygen consumption of the worm after the crown has been amputated. The crown is therefore a respiratory organ.

During the regeneration of fragments of *Sabella* the oxygen consumption slowly increases from a low initial value to that of the intact tubeless worm.

An X-ray investigation of slowly cooled copper-nickel-aluminium alloys.

By A. J. Bradley and H. Lipson. (*Communicated by W. L. Bragg, F.R.S.—Received* 13 *May* 1938.)

From an X-ray examination of slowly cooled CuNiAl alloys, a phase diagram has been constructed. The boundaries of the single-phase, two-phase and three-phase fields have been fixed from powder photographs. A novel feature of the work is the use made of the relative intensities of the X-ray reflexions to estimate the proportions of the constituents in two- and three-phase alloys.

The single-phase fields are derived from the copper-aluminium and nickel-aluminium systems, with the exception of (Cu_3NiAl_6), which has a new type of structure, derived from a deformed body-centred cube.

The molecular structures of carbon and silicon tetrafluorides.

By C. R. Bailey, J. B. Hale and J. W. Thompson. (*Communicated by C. K. Ingold, F.R.S.—Received* 13 *May* 1938.)

The infra-red absorption spectra of the vapours of CF_4 and SiF_4 have been examined with a prism spectrometer between 1 and 18μ. The results are compared with those for the Raman effect as obtained by other workers. Certain molecular constants are determined, and it is found that a force field of the type devised by Urey and Bradley fits the observations very closely indeed. The internal electrical polarity of such tetrahedral pentatomic molecules is discussed with particular reference to the type of bonding, and to the interatomic distances as obtained by different methods.

The measurement of sand storms. By R. A. BAGNOLD. (*Communicated by G. I. Taylor, F.R.S.—Received* 16 *May* 1938.)

Measurements are described of wind velocity and sand movement during storms in the Egyptian Desert. The results are shown to confirm in the following respects the author's previous laboratory (wind tunnel) work:

(*a*) The modification of the normal wind velocity distribution caused by the drag of the surface sand movement.

(*b*) The relation between the rate of flow of sand over a dune and the wind gradient required to drive it.

(*c*) The particular form taken by the grading diagram of sand which has been picked up by the wind and is in process of transport over the surface.

A special multi-tube manometer is described which was designed for operation on the open surface of a dune during a sand storm.

Selective neutralization of the luteinizing activity of gonadotrophic extracts by antisera. By I. W. ROWLANDS. (*Communicated by A. S. Parkes, F.R.S.—Received* 16 *May* 1938.)

Experiments on immature female rats show that it is possible to change the qualitative activity of gonadotrophic extracts of the pituitary by the simultaneous administration of anti-gonadotrophic sera. Two extracts of horse pituitary were used, both of which alone produced large numbers of corpora lutea in the immature ovary. Two antisera were prepared by injecting batches of rabbits daily for periods of 3 and 5 months respectively with gonadotrophic extracts of ox pituitary.

It was found that with suitable doses of antiserum the luteinizing activity of the extract was completely inhibited, although the response measured by increase in weight of the ovary was only slightly altered. Histologically, the response was one of pure follicle stimulation, as shown by the presence of numerous large follicles, and the absence of corpora lutea or any luteinization of the membrana granulosa. The uterus of all these animals was greatly distended with fluid such as is found in the normal animal during oestrus. Since these effects were not produced by variation in the dosage of horse pituitary extract alone, they cannot be due to a partial quantitative neutralization of the extract by the serum. When larger amounts of antiserum than the optimum were given there was a progressively increasing inhibition of follicular stimulation and of the ovarian weight response.

The significance of these results is discussed in relation to the probability that separate follicle stimulating and luteinizing hormones are present in pituitary extracts.

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable. The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form. These abstracts will be issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings".

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Diffusion of electrolytic hydrogen through metals. By H. R. HEATH and T. L. IBBS. (*Communicated by M. L. Oliphant, F.R.S.—Received* 20 *May* 1938.)

Experiments were made to test whether hydrogen produced electrolytically on one side of a metal plate will diffuse through the plate. The Shakespear katharometer was used to detect and measure the diffused gas. The metals examined were aluminium, iron, nickel, cobalt, copper, zinc, silver, palladium, platinum and lead. Only in the case of iron and palladium was any diffusion observed.

Observations were also made with iron showing how the amount of diffused gas varied with the time, and how the rate of diffusion varied with the thickness of the plate.

The variation of the rate of diffusion with the electrolysing current density was observed for plates of iron and palladium. The results agree well with the conclusions of Borelius and Lindblom for large current densities; but show that a threshold value of current density, below which no diffusion occurs, does not really exist.

The diffused gas appears to be in the ordinary diatomic state. The absorption of electrolytic hydrogen by palladium was also studied.

The band spectrum of lead fluoride. II. By G. D. Rochester. (*Communicated by W. E. Curtis, F.R.S.—Received* 21 *May* 1938.)

An account is given of the emission and absorption band spectra of PbF. Six new systems and two continua are found. The vibrational constants of the stable electronic states of PbF are as follows in cm.$^{-1}$:

State	Probable type	ν_e	ω_e	$x_e\omega_e$	Observed in emission (E), absorption (A)
X_1	$^2\Pi$	0	507·2	2·30	E, A
X_2		8266	531·1	1·50	E
A	$^2\Delta$	22567	397·8	1·77	E, A
B	$^2\Sigma$	35643	612·8	3·42	E, A
C	$^2\Delta$	38046	594·0	2·50	A
D	$^2\Pi$	43820	600·0	—	A
E	—	45400	565·0	—	A
F	$^2\Pi$	47870	630·0	—	A
	2440 A continuum		maximum	41000	A
	3050 A continuum		maximum	32800	E

The width of the ground state, i.e. 8266 cm.$^{-1}$, is the largest yet recorded. Numerous examples of predissociation are found.

Studies on the ionosphere at Allahabad. By B. D. Pant and R. R. Bajpai. (*Communicated by M. N. Saha, F.R.S.—Received* 23 *May* 1938.)

In this paper are described the results of study of the ionosphere at Allahabad from October 1936 to March 1938. Various types of reflexions, including complex echoes, are described and illustrated. A typical curve showing the diurnal variation of electron density is given. The results of measurements of the critical penetration frequencies for the *O* and *X* waves show that at times the *X* wave gets reflected from a layer where the electron concentration is given by the condition $p_0^2 = p^2\dfrac{p^2-p_h^2}{p^2-p_L^2}$ instead of the usual condition $p_0^2 = p^2 - pp_h$.

A new Coriolis perturbation in the methane spectrum. I. Vibrational-rotational Hamiltonian and wave functions. By H. A. Jahn. (*Communicated by Sir William Bragg, O.M., P.R.S.—Received* 25 *May* 1938.)

The theoretically possible sub-levels into which the rotational levels of a threefold degenerate mode of vibration of methane can be split by a tetrahedrally symmetrical perturbation are given up to the tenth rotational quantum number together with the correct vibrational-rotational wave functions with which to carry out the perturbation calculation. Good approximations to the true modes of vibration of the methane molecule are found and used to evaluate certain Coriolis terms in the

Hamiltonian which couple the twofold and threefold degenerate modes of vibration. Table III which gives the tetrahedrally irreducible harmonic functions and extends calculations of Bethe (1929) from the fourth to the tenth quantum number will be useful in a number of connections.

Nature and causes of the adaptation of water-breathing creatures to air breathing. I. The bionomics structure and physiology of *Lepidocephalus*.

By B. K. Das. (*Communicated by E. W. MacBride, F.R.S.—Received 25 May* 1938.)

This paper gives an account of the habits and structure of the fresh-water fish *Lepidocephalus*. The aim of the author is to show that the structure of the air-breathing organs depends entirely on the place in the body where the air bubbles are trapped. Thus in previous papers he had shown that the air-sac in *Anabas* is a part of the opercular cavity whereas in *Amphipnous cuchia* and in Ophiocephalidae there exist a pair of pouches of the pharynx which contain air. In *Lepidocephalus*, with which the present paper deals, a part of the intestine is converted into an air-breathing organ. It is supplied with a rich blood plexus from which a large vessel is given off to the portal vein. The fish lies near the surface of the ponds and streams in which it lives and from time to time rises in order to swallow bubbles of air.

Thermal effects on bodies in an air stream.

By W. F. Hilton. (*Communicated by E. F. Relf, F.R.S.—Received 26 May* 1938.)

A theoretical expression derived from Pohlhausen's formula, giving the temperature due to laminar flow over a flat plate, has been tested experimentally in the one-foot diameter high-speed wind tunnel at the N.P.L. The temperatures were measured by using thermocouples, and good agreement between theory and experiment was obtained with the thermocouple near the leading edge of the flat plate, where the flow was probably laminar. A 26% discrepancy was found with the thermocouple near to the trailing edge, due probably to the flow being turbulent.

On all models tested the temperature difference was found to increase as the square of the wind speed, until a shock wave was formed, which gave an inflexion in the δT, V^2 curve. The present method can thus be used to detect the formation of a shock wave at a solid boundary, such as an aerofoil, or aeroplane body.

Besides tests on flat plates of four thicknesses, and a streamline bar at four incidences, measurements were made on a circular cylinder, and two other shapes. The circular cylinder was found to be a most unsatisfactory shape for the thermometry of moving fluids, unless placed along the wind. A flat plate with a thermocouple near the leading edge is suggested to be the best shape for a sensitive element.

Photochemical reactions in monolayers. II. The photochemistry of proteins.

By J. S. Mitchell and E. K. Rideal, F.R.S. (*Received* 26 *May* 1938.)

This paper is an account of a quantitative investigation of the photochemistry of proteins in monolayers. Wheat gliadin, zein, insulin and ovalbumin have been examined. The photo-reactions were studied by observation of the changes in phase boundary potential of the protein monolayers on irradiation, at constant surface area and under equilibrium conditions, with monochromatic ultra-violet light of wave-lengths 2350–3175 A.

The general theory of photochemical reactions in protein monolayers is summarized. The proteins in the monolayers are to be regarded as macromolecular polycondensation products in which the primary photochemical processes are limited, to a first approximation, to the "aromatic" chromophores.

The experimental technique, including the method of fractional irradiation, and the preparation and properties of the monolayers are described.

The characteristic changes on ultra-violet irradiation of protein monolayers are:

(1) Liquefaction, if the film is gelatinous.

(2) At constant area, an increase of phase boundary potential, and also of surface pressure.

Oxidative processes play a fundamental part. Photosensitization, probably by traces of metals, is described.

It is suggested that, in these photoreactions, both "peptide linkages" adjacent to each side chain derived from tyrosine, phenylalanine and possibly also tryptophane may undergo a process of oxidative photolysis, the end result of which is equivalent to hydrolysis, and the transformed chromophoric residues liberated pass into solution. This hypothesis is in quantitative agreement with the total changes in phase boundary potential observed on irradiation of the monolayers of gliadin, zein and ovalbumin (the insulin films undergo partial solution). The importance of the Hardy-Langmuir principle is demonstrated.

The absolute quantum efficiency of the total changes per aromatic residue in monolayers of gliadin on N/100 sulphuric acid is of the order of $\frac{1}{4}$ for unsensitised photodecomposition at wave-lengths between 2350 and 3030 A.

The subthreshold potentials in a crustacean nerve fibre.

By A. L. Hodgkin. (*Communicated by E. D. Adrian, F.R.S.—Received* 26 *May* 1938.)

Experiments with isolated crustacean nerve fibres show that subthreshold shocks produce two quite distinct effects. When the stimulus is weak and cathodic, or anodic and of any strength, it produces a "polarization" potential which behaves as though it were due to the passive accumulation of charge at the nerve membrane. On the other hand, when the stimulus approaches threshold, it produces an additional wave of negativity which behaves like a subliminal response of the nerve fibre. These two processes have entirely different properties. Thus:

The size of the polarization potential is directly proportional to the strength of the shock, whereas the subliminal response increases at an accelerating rate as the shock approaches threshold.

The time relations of the processes are different. The polarization potential rises during the period in which the shock is applied and then declines in an approximately exponential way. On the other hand, the subliminal response starts with an initial inflexion and continues to grow for as much as 500μsec. after the end of the shock.

The time relations of the polarization potential are independent of the strength of the shock; whereas the duration of the local response increases and its maximum occurs later as the shock approaches threshold.

When the local response has almost reached propagating size, it becomes unstable and varies from shock to shock. On the other hand, the polarization produced by successive shocks is always quite constant.

The local response can be greatly reduced or abolished by making the nerve refractory, whereas the polarizability is only slightly reduced by refractoriness.

The local response can be increased by making the nerve supernormal, whereas the polarization potential is slightly reduced or unaffected during the supernormal phase.

The spatial spread of the two processes is different. The polarization potential falls to one-third of its value in approximately 0·5 mm., whereas the local response usually declines to only two-thirds in that distance. Under certain conditions the local response may spread very much farther than the polarization.

The latency of the nerve fibre is determined by the time relations of the local response. The latency is short when the local wave has a rapid rise and fall, whereas it is long when the wave has slower time relations. It is suggested that the latency of the fibre is due to the time required for the local response to reach propagating size.

The potential at which the local response starts to propagate is increased during the relatively refractory period and decreased during the supernormal phase. In a normal nerve the response starts to propagate when its amplitude is equal to about one-fifth of the spike. During refractoriness this large safety margin may be reduced to the point at which a propagated action potential is only slightly larger than one which just fails to propagate. Under these conditions both sub- and superthreshold waves of activity at first spread along the nerve in the same way and do not finally separate until they have travelled a short distance from the cathode.

The comparison of series of measures when the standard errors are unequal. By H. Jeffreys, F.R.S. (*Received* 27 *May* 1938.)

A test previously given for the significance of the difference of two means, when the standard errors are unequal, is revised to take account of the uncertainties of the standard errors and of a theoretical complication that has given trouble in other tests. The case where the standard errors can be assumed equal is derived from an existing formula. Some incidental points are discussed and a list is given of tests that have been revised in later work.

Statistical theory of superlattices with long-range interaction. By J. S. Wang. (*Communicated by R. H. Fowler, F.R.S.—Received* 30 *May* 1938.)

I. General Theory. Bethe's theory of order-disorder in superlattices is extended to cover the case of long-range interactions. The theory is applicable to any interaction energy of the form $V(r)$ subject to the condition $\int_0^\infty V(r)\,r^2dr =$ finite, and to a general class of lattice structures which include the three lattices of the cubic system.

II. The simple cubic lattice and the body-centred cubic lattice. The general statistical theory of superlattices with long-range interaction given in the preceding paper is applied to the simple cubic lattice and the body-centred cubic lattice. It is shown that the assumption of an inverse power law for the interaction energy alters the calculated configurational energy in the direction required to make the agreement with experiment better than the assumption of interaction between nearest neighbours only, but the shift is not great enough to remove the discrepancy between theory and experiment completely.

The androgenic activity of ovarian grafts in castrated male rats. By R. Deanesly. (*Communicated by Sir Henry Dale, F.R.S.—Received* 1 *June* 1938.)

Ovarian grafts were made into the ears of immature castrated male rats. All functional grafts showed oestrogenic activity, detectable by the fibrosis of the seminal vesicles. Most functional grafts, especially those established for three months or more, showed androgenic activity causing secretion and growth in the prostate and, occasionally, in the seminal vesicles. One rat, with a graft 135 days old, had a prostate of full adult size, though the seminal vesicles were not correspondingly large. The slighter response of the vesicular epithelium as compared with that of the prostate suggests that the androgenic substance produced by the grafts is not testosterone.

Androgenic activity does not depend on the temperature at which the rat is kept; it is associated with luteinization of the theca interna of the follicles, which occurs commonly in established grafts.

Gonadotrophic extracts which cause extensive luteinization of the granulosa do not appreciably affect the androgenic activity of the graft.

A new Coriolis perturbation in the methane spectrum. II. Energy levels. By H. A. Jahn. (*Communicated by Sir William Bragg, O.M., P.R.S.—Received* 3 *June* 1938.)

Using the vibrational-rotational wave functions derived in Part I, the perturbations of the rotational levels of the fundamental vibration ν_4 of the methane molecule, caused by the Coriolis term in the Hamiltonian which couples the rotational levels of

ν_4 with the rotational levels of ν_2, are calculated explicitly up to the tenth rotational quantum number. Table III, which gives the wave functions for the tetrahedral rotational sublevels of ν_2, will be useful in calculating the perturbations of the rotational levels of any fundamental or overtone subvibrational level of symmetry type *E* in a tetrahedrally symmetrical molecule.

Photographic sensitivity and the reciprocity law at low temperatures.

By W. F. BERG and K. MENDELSSOHN. (*Communicated by F. A. Lindemann, F.R.S.—Received* 3 *June* 1938.)

Experiments are described which were undertaken to test a theory on the formation of the latent image by Gurney and Mott which differs from earlier theories in stressing the importance of movements of silver ions. It is shown that photographic materials have an appreciable sensitivity down to 20° K. While the sensitivity drops by 93% by changing from room temperature to 90° K., very little change occurs between 90 and 20° K. The main difference between exposure at 90 and 20° K. is a drop in contrast and in maximum density. It is shown that no reciprocity failure takes place at these temperatures. It appears that at sufficiently low temperatures the latent image is formed in two distinct stages: an electronic process, in which the electrons are separated from the halide ions absorbing the light, and are trapped in the lattice, and an ionic process which takes place on warming up, and consists of a movement of silver ions and probably also of bromine atoms. These results confirm the importance of ionic movements for the latent image formation.

Relaxation methods applied to engineering problems. III. Problem involving two independent variables.

By D. G. CHRISTOPHERSON and R. V. SOUTHWELL, F.R.S. (*Received* 8 *June* 1938.)

In this paper relaxation methods are applied with success to four problems involving Laplace's, Poisson's and similar equations in *two variables*, namely:

The torsion problem of Saint-Venant for triangular shafts (this, since the solution is known, serves to illustrate and in some degree to test the methods);

The same problem for a shaft pierced by axial holes (a multiply-connected cross-section);

The torsion problem modified by the imposition of a definite limit to the shear stress (Prandtl's problem);

The problem of magnetic induction in a field containing iron.

In all four problems the methods lead without difficulty to solutions of sufficient accuracy for practical purposes, and it seems reasonable to conclude that they will be applicable (suitably modified) to other problems which, like the last three above, would present great or insuperable difficulties if treated by orthodox methods.

The nuclear spin of iodine. III. Further measurements upon the fine structure in the first spark spectrum.

By S. Tolansky. *(Communicated by P. M. S. Blackett, F.R.S.—Received* 8 *June* 1938.)

Earlier measurements by Tolansky upon the fine structures in the lines of the first spark spectrum of iodine are extended into the ultra-violet region with the aid of a quartz Lummer plate interferometer. Fine structures are now known for 58 lines, 39 of which have been allocated to terms which go either to the (^{4}S) or the (^{2}D) series limit.

An independent fine structure analysis is made for both the systems and is confirmed by the aid of intercombination lines in the ultra-violet. Fine structure interval factors are given for 13 (^{4}S) terms and 15 (^{2}D) terms. These exhibit anomalies which can be explained as arising from configuration interaction perturbation.

It is shown that the nuclear coupling of the $5p^3$ electrons in iodine is comparable with that of the $6s$ electron.

In three terms, deviations from the interval rule have been established. These are attributed to nuclear electrical quadrupole moment. The perturbation constants have been calculated, but these cannot as yet be employed to give a numerical estimate of the quadrupole moment, since the coupling constants have not yet been evaluated.

Experiments on the transmutation of fluorine by protons and deuterons.

By W. E. Burcham, C. L. Smith and P. I. Dee. *(Communicated by E. V. Appleton, F.R.S.—Received* 8 *June* 1938.)

The transmutations of fluorine under bombardment with artificially accelerated protons and deuterons have been systematically investigated.

The energy of the group of α-particles which is emitted during bombardment of fluorine with protons has been accurately determined and leads to a value 19·0043 for the mass of ^{19}F, in good agreement with the mass spectroscopic value. From the value of the energy release in this process and from the absence of α-particle groups of smaller energy it is concluded that the α-particle emission from fluorine under proton bombardment arises from successive transitions of excited ^{20}Ne nuclei.

Five groups of α-particles were found to be emitted during bombardment of fluorine with deuterons, and the energies of these groups have been determined. From the energy values of these groups it is concluded that excitation levels at 0·83, 2·95, 3·77, and 4·49 × 10^6 e-volts above the ground state exist in ^{17}O nuclei.

The emission of a group of protons which is believed to be associated with the formation of ^{20}F has been observed. From the energy of these protons the value 20·0087 has been deduced for the mass of radioactive ^{20}F. An energy release of 9·2 × 10^6 e-volts is therefore to be expected in the radioactive decay process

$$^{20}\mathrm{F} \rightarrow {}^{20}\mathrm{Ne} + e^-$$

and the disagreement between this value and the observed end-point energy of the β-rays (5·2 × 10^6 e-volts) has been partially reconciled by the observation that a radioactive emission of γ-radiation accompanies the β-emission and decays with the same period.

ABSTRACTS

OF PAPERS COMMUNICATED TO THE ROYAL SOCIETY OF LONDON

In accordance with a resolution of Council, summaries or abstracts of papers are to be published as soon as practicable. The publication of such abstracts in no way indicates that the papers have been accepted for publication in any fuller form. These abstracts will be issued for convenience with the "Proceedings of the Royal Society of London" but do not form a part of the "Proceedings".

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5 AUGUST 1938

A study of the chain reaction in the thermal decomposition of diethyl ether.

By J. E. HOBBS. (*Communicated by C. N. Hinshelwood, F.R.S.—Received 8 June* 1938.)

The course of the curves showing the rate of decomposition of diethyl ether as a function of minute additions of nitric oxide is independent of the concentration of the diethyl ether. The removal of a radical by the nitric oxide must thus be in competition with some reaction the rate of which is entirely independent of the concentration of the ether. This must be a unimolecular dissociation of the radical itself. This is in contrast to the inhibition of the decomposition of ethane where nitric oxide removes a radical which would otherwise have reacted with a molecule of ethane. A chain mechanism explaining these results is the same as that put forward by Rice and Herzfeld to explain the first order nature of the reaction. The mechanism gives an expression for the shape of the inhibition curve and accounts for the variation of the mean chain length with the ether pressure. From the amount of nitric oxide required for a given degree of inhibition the average life of the radical can be calculated to be of the order of $1 \cdot 2 \times 10^{-5}$ sec.

An analysis of the mechanism of oestrogenic activity.

By G. PINCUS and N. T. WERTHESSEN. (*Communicated by F. H. A. Marshall, F.R.S.—Received 8 June* 1938.)

To facilitate the study of a series of thirty synthetic oestrogenic substances a new method for the bio-assay of the materials in question was advised. Mice were used as the test animals. The substances were dissolved in oil and administered intraperitoneally. The value obtained by assay had an error of approximately 10 %. A comparison between the time course of action for oestrone and the other oestrogens was possible. It was found that the hydroxyl group contributes quantitatively to the oestrogenic activity. Structure, when approximating to that of natural hormones by adding the five-membered ring to the phenanthrene nucleus, also contributes to the activity. Activity is generally low—the maximum being 1·72 % that of oestrone, Subcutaneous injection decreases the activity value many times. Oestrone and oestradiol in adequate doses show a definite difference in the time course of action from oestrone. The synthetic oestrogens do not. Simultaneous injection of several synthetic oestrogens with progesterone into rabbits ovariectomized after fertile copulation stimulated the progesterone effects. This was also found in the case of small doses of the native oestrogens. The synthetic agents are not progestins. Oestrone in higher doses inhibits both the progesterone induced proliferation of the uterus and also the growth of the eggs. Oestradiol and oestriol inhibit, when given in large doses, the proliferation of the uterus but do not affect the growth of eggs.

Problems on stability in hydrophobic colloidal solutions. I. By S. Levine.

(*Communicated by E. K. Rideal, F.R.S.—Received* 13 *June* 1938.)

A method of obtaining the mutual electrical energy of two colloidal hydrophobic spherical particles has been developed by applying the Debye-Hückel theory of electrolytes. The final result is

$$\frac{Q_0^2}{Da} f\left(\frac{R}{a}, \kappa a\right) = \zeta^2 Da(1+\kappa a)^2 f\left(\frac{R}{a}, \kappa a\right),$$

where Q_0 is the charge on each particle, assumed to be constant, a its radius, ζ its potential, R the distance between the particles, D the dielectric constant of water and κ is the well-known Debye-Hückel constant, being proportional to the square root of the electrolytic concentration. Only the approximate Debye-Hückel equation has been considered.

The electrical forces are repulsive at small separations but become attractive at large, passing through a zero at some intermediate distance, where the energy is a minimum. The energy near contact decreases very rapidly with increase in electrolytic concentration even though the charge does not change. This suggests that the diminution of the mutual energy as electrolyte is added is chiefly due to the behaviour of the ionic atmospheres, the effect of the adsorption of ions tending to lower the charge being less important.

There are two distinct terms in the energy function. The first represents the repulsion that one particle experiences when it enters the field of a second particle, and is the more important term at small separation. The second term represents the attraction that is exerted upon the ionic atmospheres when overlapping occurs and is the dominating contribution at large distances.

The van der Waals attractive energy for a particular case is computed. The resultant total energy function exhibits a maximum at a little distance out from contact. The value of the energy at this maximum is the controlling factor in coagulation, its value diminishing with electrolytic concentration.

The theory suggests an explanation for the fact that with electrolytes which coagulate at low concentrations the critical potential is much lower than with electrolytes which precipitate at large concentrations.

The Schultz-Hardy rule is discussed. It is shown that the use of the approximate Debye-Hückel equation cannot explain this rule for the ions of higher valency and it is necessary to consider the complete equation.

The energy minimum is characteristic of all hydrophobic sols containing spherical particles, and, contrary to current belief, the energy is always negative at large distances. Whereas the van der Waals forces cannot be of a long range character, the electrical forces can yield a minimum in the energy at a distance of several particle radii and the minimum energy may be a small multiple of kT. In general, it is likely that, at least for hydrophobic sols, thixotropy and related phenomena are mainly due to the existence of this minimum in the electrical energy and not to the van der Waals energy.

The method of calculating the osmotic pressure from the expression for the interaction energy is indicated.

Problems of stability in hydrophobic colloidal solutions. II. By S. Levine. (*Communicated by E. K. Rideal, F.R.S.—Received* 13 *June* 1938.)

The mathematical method of deriving the mutual energy of two colloidal metallic particles is developed. Only a first and second approximation are obtained. In the appendix a statistical analysis of the free energy evaluated here is given. It is seen that the Debye-Hückel theory of electrolytes can be applied much more satisfactorily to colloidal solutions than to ordinary electrolytic solutions. This is true even if the electrolyte present in the sol is of an unsymmetrical type.

On the absorption of light by crystals. By N. F. Mott, F.R.S. (*Received* 14 *June* 1938.)

The mechanism of the absorption of light by solids is discussed from the theoretical point of view. The energy of a quantum of radiation can be converted into heat, re-emitted as fluorescent radiation, or used up in producing a photoelectric current within the solid; estimates are made of the dependence on temperature of the relative probabilities of these processes, which are compared with experiment.

The thermal properties and heats of adsorption of films on vitreous silica. By W. G. Palmer. (*Communicated by Sir William Pope, F.R.S.—Received* 14 *June* 1938.)

Isothermals have been obtained at temperatures between 70° and 25° for adsorption of the vapours of benzene, acetone and methyl alcohol on known surfaces of vitreous silica, by methods giving a check on the constancy of the surface condition. The values of the coefficients of thermal expansion, compressibility and the temperature variation of the lateral force have been calculated and discussed in relation to parallel properties of the bulk phase. The heats of adsorption have been calculated from the temperature variation of the adsorption potential. Their relations to the fraction of surface covered are in agreement with theoretical predictions for films with interaction energy.

The electrical conductivity of the transition metals. By A. H. WILSON. (*Communicated by R. H. Fowler, F.R.S.—Received* 20 *June* 1938.)

By using a model in which the energy surfaces are spheres, a complete theory is worked out of the *s-d* transitions in a metal containing incomplete *d*-bands. The results are of the same order of magnitude as those given by Mott's qualitative theory, but differ considerably in detail and in interpretation. It is shown that the *s-d* transitions should fall off exponentially as the temperature is lowered. A suggestion is put forward to explain the change in sign of the thermoelectric power of platinum at low temperatures.

The posterior probability distribution of the ordinary and intraclass correlation coefficients. By H. JEFFREYS, F.R.S. (*Received* 21 *June* 1938.)

The posterior probability distributions of the ordinary and intraclass correlation coefficients are obtained from an analogy with sampling theory, suggested by the use of the normal correlation surface in both cases. The results cast some further light on the relation between direct and inverse methods in problems of estimation; for practical purposes the differences from the recommendations made by Fisher are inappreciable. A significance test is obtained for the difference between an intraclass correlation and zero, and the application to internal correlation in series of measures is discussed.

An electrical theory of nerve impulse propagation. By O. H. SCHMIDT. (*Communicated by A. V. Hill, Sec.R.S.—Received* 22 *June* 1938.)

A capacitative theory of the nerve impulse is offered which supposes the local excitatory process to consist largely in an increase of capacitance rather than exclusively in a resistance change. This theory is shown to satisfy all requirements in respect of structures, energy, and electrical properties.

An electrical model is developed and discussed which permits direct determination of the electrical constants involved in this or other electrical theories of excitation. The results obtained from the model are shown to resemble those from nerve.

A possible physico-chemical theory of nerve excitation is developed on the basis of the capacitance-shift hypothesis.

A new process of negative ion formation. IV. By F. L. ARNOT and C. BECKETT. (*Communicated by H. S. Allen, F.R.S.—Received* 22 *June* 1938.)

The three previous papers of this series described a new process of negative ion formation. This process is the formation of negative ions at metal surfaces by bombardment of the surface with positive ions, the negative ion being formed by the positive ion capturing two electrons from the surface. The present paper shows that a new modification of this process can also occur.

The experimental results show that a spectrum of negative ions is obtained when a surface is bombarded with positive ions of argon or nitrogen or mercury, and that practically identical negative ion curves over the range of mass numbers from 10 to 54 are obtained by each type of positive ion. The negative ions observed have mass numbers 12, 16, 24, 29, 32, 38, 44 and 51. It is concluded that these negative ions arise from atoms and molecules of gas and vapour adsorbed on the surface.

It thus appears that there are two processes leading to the formation of negative ions at surfaces. The initial stage in each process is the same; this is capture of an electron from the surface by the incident positive ion into an excited state of the atom, probably by a resonance process. This excited atom, on reverting to its normal state, may then capture a second electron from the surface, again probably by a resonance process, so forming a negative ion of the same element as the incident positive ion. The second process consists in the excited atom transferring by a collision of the second kind its excitation energy to an adsorbed atom on the surface, which then captures an electron from the surface, and so a negative ion of an element different to that of the incident positive ion is produced.

The previous results are reviewed in the light of this new work which shows that no negative ions of nitrogen are formed. Certain alterations in the interpretation of the CO_2 results must also be made. The previous work on the formation of negative ions of mercury, hydrogen and oxygen requires no amendment, except that the probabilities of formation of these ions may have to be raised.

It is shown that no O_2^- ions are formed at the surface of an oxidized tungsten filament, but that they readily appear when the filament is cleaned by flashing. It is suggested that this effect is due to the increased work function of the filament when oxidized. It is also found that a covering of oxygen on the filament suppresses the peaks of mass numbers 24 and 38.

New systems of normal co-ordinates for relativistic optics. By G. Temple.

(*Communicated by Sir Arthur Eddington, F.R.S.—Received* 22 *June* 1938.)

This paper developes the theory of a new system of normal co-ordinates (y^μ) in an n-dimensional manifold with an indefinite metric of signature $+1-(n-1)$. In this system of co-ordinates the expression for the metric has the form

$$ds^2 = h_{jk}dy^j dy^k + 2dy^{n-1}dy^n, \quad (j, k = 1, 2, \ldots, n-1).$$

Any arbitrary time-like curve C being taken as the base curve, the forward null cone at any point A on C is given by $y^{n-1} = \text{constant}$, C itself is given by the single equation $y^n = 0$, and any forward null geodesic G from A is given by $y^j = \text{constant}$ $(j = 1, 2, \ldots, n-1)$. y^n is the "projected length" defined in a general co-ordinate system (x^μ) by

$$y^n = \int K_\mu dx^\mu,$$

where the integral is taken along G from A, and K_μ is a unit vector transported along G by parallel displacement and forming the tangent to C at A.

Hadamard's theory of null geodesics as bicharacteristics leads to a generalization of this system of normal co-ordinates in which $y^{n-1} = \text{constant}$ is the equation of a system of wave fronts, $y^j = \text{constant}$ are the equations of the orthogonal null geodesics or rays, and y^n is a projected length measured along the rays.

The theory is applied to optical problems in general relativity. Fermat's principle of minimum time is established rigorously for any relativistic manifold. The discussion of astronomical determinations of distance is simplified by the use of the new system of normal co-ordinates, which gives explicit expressions for the various distance functions. These general results are illustrated by the case of a manifold representing an isotropic expanding universe. Incidentally the use of normal co-ordinates of Riemann's type in relativistic optics is justified by a new definition in terms of "projected distances".

A new Coriolis perturbation in the methane spectrum. III. Intensities and optical spectrum.

By W. H. J. Childs and H. A. Jahn. (*Communicated by Sir William Bragg, P.R.S.—Received* 22 *June* 1938.)

Using the Coriolis perturbations evaluated in a previous paper, a theoretical envelope for the infra-red absorption band of the low-frequency fundamental ν_4 of the methane molecule is calculated and compared with the experimental envelope of Nielsen and Nielsen and also with that of a simple overtone band of methane in the photographic infra-red. Theoretical intensity factors for the methane molecule which take into account correctly the spin weights of the equivalent hydrogen nuclei are also given. It is emphasized that Coriolis perturbations will in general have an important effect on the spectra of all polyatomic molecules having a threefold or higher axis of symmetry.

Representative algebra and internuclear vibrations.

By A. C. Menzies and K. Weissenberg. (*Communicated by C. G. Darwin, F.R.S.—Received* 23 *June* 1938.)

In this paper is described a simplified treatment of representation algebra; designed to facilitate the application of representation theory to wave and quantum mechanics. The method is quite general. Here it is used for the production of tables dealing with the spectroscopy of nuclear oscillations (infra-red and Raman effect) in molecules and crystals. The paper is divided into two main parts. Part I contains a new invariant treatment of the algebra of unitary representation of a given symmetry group. We first characterize each matrix of such a representation by a set of integers which are the natural logarithms of the proper values of the matrix, multiplied by a factor $\omega/2\pi i$ (where ω is the order of the group operation). The whole representation, which is the aggregate of the matrices, is then characterized by the aggregate of the corresponding sets of integers, which we call "mode numbers" (abbreviated to m.n.). Representation algebra is thereby reduced to the arithmetic of simple integers, and rules are given which make possible a drastic reduction in the number of m.n. required. In application to wave mechanics the m.n. are simply related to quantum numbers of angular momentum type. Part II gives in the form of explicit tables the results obtained from the application of this treatment to the spectroscopic problem mentioned above. The nuclear oscillations are grouped into the various normal modes which are distinguished from one another by the m.n. of their corresponding representations, so that the m.n. serve as a convenient nomenclature for the modes. In the tables are listed the fundamental modes and the fundamental oscillations and properties associated with each of them. By applying the algebra of Part I to the m.n. characterizing these fundamental modes, the properties of obertones and combinations are calculated.

The decomposition and ignition of peroxides. I. Diethylperoxide. By A. C. Egerton, F.R.S., and E. J. Harris. (*Received* 24 *June* 1938.)

The decomposition of diethylperoxide in the range 130–190° C. occurs in a unimolecular manner and is very little affected by surface or by diluents. The rate is given by $\log_{10} K = -6890{\cdot}4/T + 14{\cdot}708$ with an apparent activation energy 31500 cal. However, as first shown by Neumann and Toutakin, above a certain critical pressure the reaction becomes explosive; this effect has been studied, and the critical pressure is found to depend on the diameter of the vessel and to a less extent on the presence of diluents; the results do not accord with the simpler forms of theory for thermal explosions, and further experiments are being made.

The ignition of the peroxide is related to its explosive decomposition, the slope of the $\{\log p/(I/T)\}$ curve being the same in a similar vessel; but the ignition takes place at a somewhat lower temperature than corresponds to the critical explosion pressures, owing to the increase in the exothermicity. Ignition of diethylperoxide can occur in propane oxygen mixtures without ignition of the propane; it is possible therefore that substances formed in slow oxidations of hydrocarbons may behave like diethylperoxide and give rise to cool flames or to cool flames succeeded by true ignition. Diethylperoxide greatly diminishes the induction period of the slow combustion of propane.

The dissociation of the ammonium ion and the basic strength of ammonia in water. By D. H. Everett and W. F. K. Wynne-Jones. (*Communicated by Sir Harold Hartley, F.R.S.—Received* 24 *June* 1938.)

From measurements of the E.M.F. of cells comprising hydrogen electrodes in buffer solutions of ammonia and ammonium chloride the dissociation constants of the ammonium ion in water have been determined at different ionic strengths at 5, 15, 25, 35 and 45°. By combination with the data of Harned for the ionic product of water, these results give values for the ionization constants of ammonia. It is found that the dissociation of the ammonium ion varies with the temperature according to the equation:

$$-\log K = 2706/T + 0{\cdot}139,$$

corresponding to a heat of dissociation of 12,400 cal. The ionization of water and of ammonia, however, do not vary with the temperature in such a simple way and their heats of ionization decrease with rising temperature.

The difference in the behaviour of these substances is regarded as the consequence of the varying electrical type of the dissociation process. If ions are produced in a reaction, the orientation of solvent molecules by the ions causes an entropy decrease and also a decrease in the heat capacity, and therefore a changing heat of ionization. Where the dissociation process results in no change in the number of ions, or, more correctly, of charge centres, the heat of dissociation is practically constant.

On the corpora allata of dipterous insects. Part II. By E. T. Burtt. (*Communicated by A. D. Imms, F.R.S.—Received* 27 *June* 1938.)

Evidence is given concerning the function of Weismann's ring which, as was suggested by the author in 1937, represents in *Calliphora* the corpora allata of other insects. Methods are described for extirpating Weismann's ring in the larva of *Calliphora*. Pupation can be prevented either by removing Weismann's ring or by cutting the nerve connexions to this organ. Removal of the ring in larvae which are still feeding causes an arrest of development. Such larvae continue to feed for a period greatly exceeding the normal, and the growth of their imaginal buds is arrested. It is concluded that Weismann's ring secretes the pupating hormone discovered by Fraenkel, and in addition influences processes taking place prior to pupation. The work of Hadorn on Weismann's ring in *Drosophila* is briefly reviewed, and the somewhat discordant results obtained by workers who have removed the corpora allata in species of Orthoptera and Lepidoptera are discussed.

The low temperature properties of gaseous helium. By R. A. Buckingham and H. S. W. Massey. (*Communicated by J. E. Lennard-Jones, F.R.S.—Received* 27 *June* 1938.)

The viscosity and second virial coefficients of helium gas are calculated in the temperature range 0–26° K, using exact quantum formulation and the interatomic interaction given by Slater. It is found that there is very good agreement with the observed viscosity down to 15° K, but below this temperature the calculated values are too small. The correct form for the variation of the virial coefficient with temperature is obtained but the calculated behaviour is in error in predicting too low temperatures for the position of the maxima, etc. (The calculated Boyle point is 21° compared with the observed 23°.)

It is shown that the discrepancies may be greatly reduced in both cases by small modification of the Slater field involving reduction of the van der Waals force at large distances and inclusion of dipole-quadripole interaction. No difference between the theoretical results obtained by assuming different statistics is sufficiently definite to be distinguished from small errors in the assumed interaction, in the temperature range from 1° K upward.

The separation of isotopes for the investigation of nuclear transmutations. By E. LEIGHTON YATES. (*Communicated by E. V. Appleton, F.R.S.—Received* 27 *June* 1938.)

A mass spectrograph of simple design has been used to prepare deposits containing a few micrograms of the separated isotopes of lithium, boron and carbon. In the case of lithium, a filament type ion source was employed, while the boron and carbon ions were produced in a low voltage capillary arc running in boron trifluoride and in a mixture of heavy methane and helium respectively. On examination in artificial disintegration experiments, the samples were found to be of high purity.

Optical pyrometry. II. By F. A. CUNNOLD, A. C. EGERTON, F.R.S., and M. MILFORD. (*Received* 28 *June* 1938.)

The accuracy of the disappearing pyrometer as a means for determining high temperatures is emphasized; the sensitivity is such that temperature changes of about 1/5th of a degree can be detected. The simple means of determining the gold point described in *Proc. Roy. Soc.* A, **130**, 111, 1930, have been improved and an error due to the lack of equilibrium between the wire and the platinum tube eliminated by increasing the absorption coefficient of the wire. It is shown, as a development of the expression derived by Foote, that the effective wave-lengths used in the calibration of the pyrometer in terms of the current through the filament are those for which the temperature values are the brightness temperatures of the pyrometer filament.

The use of sectors in the calibration of the pyrometer is discussed, and greater accuracy can be obtained by means of a wide angle sector applied several times, rather than by using a single sector of narrow angle.

The best form in which to express the results of the calibration is discussed. The formula for one particular lamp used being:

$$T = a + bV^{-1} + cV,$$

where $a = +1224{\cdot}913$, $b = -288{\cdot}002$, $c = +417{\cdot}191$. The measurement of the effective wave-lengths of the special monochromatic colour filters used and of the transmission coefficients of the neutral absorbing glasses is described and the errors in the final extrapolation to very high temperatures determined.

The kinetics of electrode reactions. By J. N. AGAR and F. P. BOWDEN. (*Communicated by R. G. W. Norrish, F.R.S.—Received* 28 *June* 1938.)

Part I. The effect of local concentration changes on overpotential. We can distinguish three main causes of irreversibility in electrode reactions. These are:

(i) Concentration overpotential.
(ii) Activation overpotential.
(iii) Resistance overpotential.

A general theory of concentration overpotential is developed, and this shows how the overpotential will depend on the current density, concentration and temperature. An expression for the rate of growth of concentration overpotential after switching on the current is also given.

It is shown that the resistance of the diffusion layer may in some cases attain high values, and cause a resistive potential drop at least as great as the concentration overpotential. Finally the principal differences between concentration overpotential and activation overpotential are described.

Part II. Electrodeposition from a fused electrolyte. Measurements of the oxygen overpotential on nickel and platinum electrodes in fused sodium hydroxide have been made. The current densities used ranged from approximately 5×10^{-4} to 1 amp./sq. cm. At the highest current density the overpotential is of the order 0·2 V.

Although in some respects the observations might suggest that the overpotential is of the same kind as that observed in the deposition of OH′ ions in aqueous solutions, the evidence as a whole shows that this is not so. The overpotential in fused NaOH is due to the change in the concentration of *water* in the melt in the immediate vicinity of the electrode. A small trace of water is normally present in the fused hydroxide, and more water is produced by the anode reaction.

This view is supported by many different lines of evidence, including:

(*a*) the influence of stirring the electrolyte;
(*b*) the effect of adding small quantities of water;
(*c*) the rate of increase of the overpotential on switching on the current;
(*d*) the nature of the relation between overpotential and current density;
(*e*) the low value of the temperature coefficient;
(*f*) the fact that the overpotential is independent of the nature of the electrode.

The general behaviour is consistent with the theory of concentration overpotential developed in Part I.

On the polymerization of vinyl acetate. Part I. By A. C. CUTHBERTSON and E. K. RIDEAL, F.R.S. (*Received* 28 *June* 1938.)

Details are given for the preparation of pure vinyl acetate. Freshly distilled aldehyde-free vinyl acetate shows no appreciable reaction up to a temperature of 100° C over a period of 24 hr. It has been shown that the addition of acetaldehyde (prepared in air) to pure vinyl acetate catalyses the reaction at 100° C. Experimental data have been presented for the course of the polymerization of pure vinyl acetate catalysed by benzoyl peroxide at 60, 80 and 100° C and for the former in toluene solutions at 80° C. Chain lengths of the polymers at 60° C have been computed from viscosity data obtained from measurements made with an Ostwald viscometer which were shown to be in relative agreement with comparable measurements made with a Couette viscometer. At 80° C there is evidence that chain branching occurred particularly in the later stages of the reaction. During the reactions catalyst decomposition takes place as an independent side reaction. This effect is noticeable at 60 and 80° and is very marked at 100° C. The reactions are exothermic as a result of which there is a temperature rise during the course of the polymerizations. This rise is progressively more pronounced as the catalyst concentration is increased.

On the polymerization of vinyl acetate. Part II. By A. C. Cuthbertson and G. Dee. (*Communicated by E. K. Rideal, F.R.S.—Received* 28 *June* 1938.)

The interpretation of the experimental results described in Part I is discussed. The induction period in the polymerization is ascribed to the fact that the initiation process involves two concentive slow reactions, namely the formation and subsequent decomposition of a complex between the catalyst and monomer. A kinetic analysis is developed, on the basis of which it is concluded that the polymerization chains terminate by mutual deactivation. Correction has to be made for the decomposition of the catalyst which is found to be an important factor. The causes of the observed effect of toluene in reducing both rate and chain length are discussed, and two alternative explanations examined.

The experimental measurements of chain length are in some cases at least invalidated by the occurrence of branching, leading finally to the production of insoluble products. A kinetic treatment shows that branching is largely confined to the late stages of polymerization: this is in accord with experimental findings.

On the theory of coincidence experiments on cosmic rays. By N. Arley. (*Communicated by N. F. Mott, F.R.S.—Received* 28 *June* 1938.)

We have made further calculations on the cascade theory of showers put forward by Bhabha and Heitler and by Carlson and Oppenheimer. In § 1 the number of "slow" electrons in a shower is calculated, i.e. of electrons having an energy less than the critical energy of the shower producing material. In § 2 we discuss the fluctuations of the number of particles about the mean number. By means of a simplified model for the multiplication process it is made plausible that the Poisson formula holds for the fluctuation in small showers. In § 3 the energy spectrum of the soft component is discussed. With this energy spectrum the mean probabilities of finding one, two and so on particles emerging from a certain layer are calculated, giving the absorption curve and the Rossi transition curves. In §§ 4–6 we compare our results with experiments. *The quantitative agreement between theory and experiment for the absorption and the Rossi curves is satisfactory* both with regard to the shape of the curves and their dependence on the material. There is, however, a difficulty in understanding the Z^2 law found for the Rossi curve at small thicknesses. This is possibly explained by the simultaneous effects of a light quantum and an electron. The spread of showers from air is discussed in this connexion.

The rates of transformation in ethyl alcohol of ammonium and ethylammonium cyanates to the corresponding ureas. By Christina C. Miller and J. R. Nicholson. (*Communicated by J. Kendall, F.R.S.—Received* 29 *June* 1938.)

The rates of transformation of ammonium cyanate to urea at 16, 24 and 32° C, and of ethylammonium cyanate to ethylurea at 0, 10 and 20° C have been found over the concentration range 0·0002–0·04 M in ethyl alcohol containing 1·91 % of water, and compared with those for methylammonium cyanate at 0 and 10° C in the same solvent.

On the supposition that ammonium (or alkylammonium) and cyanate ions are the reactants, the applicability of the Brönsted-Gronwall, La Mer and Sandved theory to the results has been tested by assuming its validity and calculating the values of a, the mean distance of closest approach of the ions, required to give agreement. For all three cyanates a has been found to have a high positive temperature coefficient. A simplified form of Christiansen's equation for ionic reactions is not generally satisfied at infinite dilution, whether r the distance between colliding and reacting ions is taken to be constant or set equal to the temperature-variable a values.

The Arrhenius equation ($k = Ae^{-E_A/RT}$) is not fulfilled, except at the highest concentration. E_A and A vary in a parallel manner from one cyanate to another, and with change of concentration, temperature and solvent. An attempt has been made to explain the relative rates of transformation of the cyanates in water and ethyl alcohol at infinite dilution by supposing that the solvation of the reactants plays an important part.

The effect of androgenic substances on the growth of the teat and mammary gland in the immature male guinea-pig. By A. C. BOTTOMLEY and S. J. FOLLEY. (*Communicated by A. S. Parkes, F.R.S.—Received* 30 *June* 1938.)

In the young male guinea-pig between 150 and 430 g. body weight the teat grows isometrically with the body as a whole. This teat growth ceases after castration and is, therefore, maintained by the endocrine activity of the testes. The unsaturated androgens, Δ_5-*trans*-androstendiol, testosterone, Δ_5-*trans*-dehydroandrosterone and 17-methyltestosterone promote teat growth, Δ_5-*trans*-androstendiol being the most potent in this respect.

Data for the relative rates of teat growth under the stimulus of Δ_5-*trans*-androstendiol, Δ_5-*trans*-dehydroandrosterone, testosterone propionate and 17-methyltestosterone give good agreement with the law of simple allometric growth. The saturated androgens, *cis*-androsterone, *cis*-androstendiol, dihydrotestosterone and the unsaturated diketone Δ_4-androstendione, do not promote teat growth. Testosterone and its propionate were both more active in promoting teat growth when the volume of oil medium used for injection was decreased. Testosterone propionate was no more active than free testosterone.

The relative rate of teat growth caused by testosterone propionate was greater in intact guinea-pigs than in recently castrated ones. The activity of the androgens in promoting teat growth appears to be correlated with the presence of a double bond (Δ_4 or Δ_5) in the ring structure of the molecule, and the presence of at least one hydroxyl group in the 3 or 17 position also appears to be necessary for activity. The most active substance studied was an unsaturated 3–17 diol.

Histological examination showed the Δ_5-*trans*-androstendiol, testosterone propionate and 17-methyltestosterone stimulated the mammary ducts but caused very little alveolar development.

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